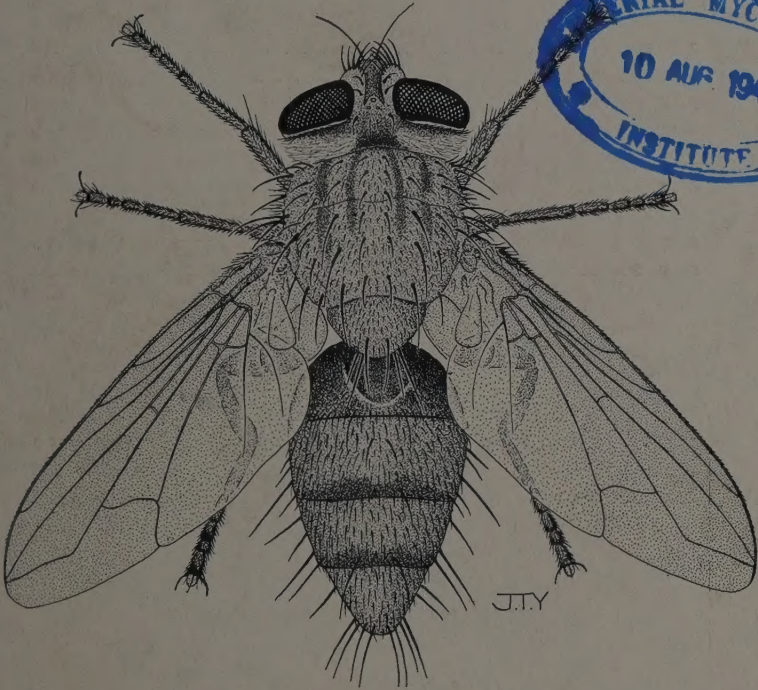# THE HAWAIIAN PLANTERS' RECORD

Eucelatoria armigera (magnified about 12 times), a beneficial tachinid fly parasitic in a variety of destructive caterpillars. Its attempted introduction here from Mexico in 1923 was unsuccessful, in the absence of air transport at the time. Now, some twenty years later, unaided, it has succeeded in reaching these Islands, probably within worm-infested tomatoes from the mainland. In the field it attacks both the corn-ear worm and the green garden looper; it is hoped it will prove effective against the nutgrass armyworm also, in which it breeds readily in the laboratory. Its life cycle, a matter of some three weeks even during the cooler months of the year, is unusually brief for an insect of this kind. During an adult life of two weeks or longer it produces 40 or more offspring. Although slightly larger than *Ceromasia*, it is liable to be confused by the casual observer with that fly enemy of the sugar cane beetle borer. The female lays maggots which are inserted within its caterpillar victim by means of a highly specialized, thorn-like larvipositor.

SECOND QUARTER 1943

HAWAIIAN SUGAR PLANTERS' ASSOCIATION

OFFICERS FOR 1943

TRUSTEES FOR 1943

EXPERIMENT STATION COMMITTEE

THE HAWAIIAN
PLANTERS' RECORD

Aconitic Acid, Calcium and Magnesium Aconitates in Sorgo and Sugar Cane Products

AVAILABLE
FOR REVIEWING

By H. A. Cook

The presence of aconitic acid in the juices of sugar-producing plants and in the products of sugar manufacture has been demonstrated qualitatively by many workers. Aconitic acid, its salts and derivatives have a commercial interest and value in the manufacture of plastics and plasticizers and they have been mentioned as having a bearing on some of the difficulties experienced from time to time in pan work and in the subsequent centrifugal separation of sugar crystals in sugar manufacture. A review of the available information on the subject is presented as a matter of local interest.

During a recent review of the literature in reference to problems and developments of particular importance to the sugar industry, a number of interesting references were found. Some of these references may have significant bearing on local problems in our own factories. Some of the references to research work showed revived interest in organic lime salts in sugar-bearing juices, particularly in aconitic acid and calcium and magnesium aconitates.

Aconitic acid, its salts and derivatives have a commercial interest and value in the manufacture of plastics and plasticizers and they have been mentioned as having a bearing on some of the difficulties experienced from time to time in pan work and in the subsequent centrifugal separation.

In view of these facts, the available information on the subject has been reviewed and brought up to date and is presented herewith as a matter of local interest.

The presence of aconitic acid in the juices of sugar-producing plants and in the products of sugar manufacture has been demonstrated qualitatively by many workers, but no very definite account of this compound in sugar juices or products has been taken until a recent publication by McCalip and Seibert (5) who made quantitative determinations of it in the sediment and scales of evaporators and pans and of its concentration in Louisiana final molasses. Behr (2) found it in molasses, in muscovado sugar, and in cane juice. Parsons (7) detected it in sorgum juice

from which, upon the addition of lime, the calcium salt separated on heating surfaces as a buff-colored, tenaciously adherent scale. Yoder (13) and Zerban (14) isolated aconitic acid from Louisiana sugar cane juice, and the former stated that it is the predominating organic acid in the juice. From the data he presented it may be calculated that he obtained approximately 0.3 per cent on the basis of dry solids. Taylor (10) found it in both healthy and diseased cane, and described the delicate color reaction it gives with acetic anhydride, a reaction which is characteristic, and was later modified by Furth and Herrmann (3). Both Taylor and Yoder noted that the calcium salt is less soluble in hot water than in cold. Prinsen Geerligs (8) found that a deposit centrifuged out of Cuban molasses contained a high percentage of calcium aconitate. Tanabe (9) recently found that aconitic acid accounted for 90 per cent of the total acid extracted with ether from a large quantity of juice from the cane variety POJ 2725. His recovery of aconitic acid may be calculated as roughly 0.1 per cent of the total solids. Nelson (6) isolated from Puerto Rican final molasses 0.8 per cent of aconitic acid, calculated on sample (equivalent to approximately 0.9 per cent on solids). Von Lippmann (11) found aconitic acid in sugar-beet products, and Beath (1) and others noted its occurrence in several species of native Delphinium and Aconitum.

Identification of the acid by these investigators was mainly through its lead, zinc, and silver salts. Reid (4) and coworkers described its p-nitrobenzyl and phenaacyl esters.

The first reference to actually accredit interference to pan and centrifugal operations to these compounds was in a report by Ventre (12):

In the present investigations, it was found that three constituents of the juices besides the sucrose content were directly concerned with its crystallization, *viz.*, starch, reducing sugars and salts of organic acids....

Some of the above prepared starch-free syrups were still found hard to work.

...crystals formed at a concentration below the degree of supersaturation necessary for sucrose crystallization. These crystals continued to grow until they reached a "smear" size and then stopped growing. When sucrose crystals were subsequently formed, these crystals remained as a "smear." They appeared to be lighter in density than the sucrose crystals as they came to the center of the centrifuge forming an impervious film preventing the massecuite from spinning.

...if the evaporator sirup was heated to 100° C. these crystals formed and readily settled to the bottom. After six to ten hours' time, as much as 10 pounds of this material per ton of cane could be separated of the consistency of wet sand. Analysis of this material indicated that it was mainly calcium aconitate with a small amount of magnesium aconitate.

The subject received extensive study by McCalip and Seibert (5):

The cream-colored sediment occurring in sirup and first and second molasses tanks during recent years in certain areas of Louisiana was studied and found to consist principally of calcium aconitate. The sediment was analyzed, and a method of separating aconitic acid from it and from related materials and of purifying it are described.

Refinery pan and evaporator scales were analyzed and found to contain aconitic acid. A simple test for the detection of aconitic acid in sediments and scales is described.

The aconitic acid content of sirups made without chemical clarification from juices from two different types of cane grown in different localities was determined and found to range from 0.75 to 1.33 per cent on solids. Two samples of Louisiana final molasses were analyzed and found to contain 1.80 and 2.52 per cent aconitic acid.

No reference or correlation was made between these results for aconitic acid and the workability of the syrups or molasses. However, they referred to the possibility that:

... sugar cane juice may prove a convenient natural source of aconitic acid, the industrial development and utilization of which has been limited because the most convenient source heretofore has been the dehydration of citric acid. The opening up of such a natural source will stimulate study of this acid, which is a derivative of both succinic and fumaric acids, and of its derivatives, many of which may readily be converted into maleic acid derivatives. Succinic and maleic acids and their derivatives are valuable intermediates in the manufacture of plastics and plasticizers.

H. P. Kortschak, of the Experiment Station staff, recently examined twenty samples of Hawaiian final molasses for insoluble crystals and reports as follows:

Twenty samples of molasses were examined microscopically for crystals insoluble in water. These were found in all but three. They were absent in one sample each from Ewa and Kohala. One Waimanalo sample was doubtful. Insoluble crystals were found in molasses from the following plantations: Honolulu Plantation, Hamakua, Honokaa, Kaiwiki, Koloa, Lihue, Maui Agric., Paauhau, Waianae, Wailuku, Waimanalo, Waimea, and Oahu.

A sample from Maui Agricultural Company contained 32 gms. of white crystals per liter separated with the super-centrifuge. Analysis showed them to consist mainly of a calcium salt of aconitic acid.

The presence of these compounds may possibly account to some extent for "scum" which is not infrequently observed in our centrifugals and at times is the cause of some difficulty in low-grade work. If it is present in our products in appreciable amounts at times, it may also have some commercial possibilities. This reopens a field of investigation that may be worthy of study.

LITERATURE CITED

(1) Beath, O. A., 1926. The Natural Occurrence of Aconitic Acid and Its Isomers. Jour. Am. Chem. Soc., 48: 2155-2158.

(2) Behr, A., 1877. Ber. Deutsch Chem. Ges., 10: 351.

(3) Furth, O., and Herrmann, H., 1935. Biochem. Z., 280, 448-457.

(4) Lyons, Edward, and Reid, E. Emmet, 1917. The Identification of Acids. III. Jour. Am. Chem. Soc., 39: 1727-1750.

(5) McCalip, M. A., and Seibert, Arthur H., 1940. Aconitic Acid from Sugar Cane Products. Ind. & Eng. Chem., 33: 637-640.

(6) Nelson, E. K., 1925. The Non-Volatile Acids of the Blackberry. Jour. Am. Chem. Soc., 47: 568-572.

(7) Parsons, H. B., 1882. Jour. Am. Chem. Soc., 4: 39-42.

(8) Prinsen Geerligs, H. C., 1933. Voorkomen Van Aconietzuur in Rietsuikerproducten. Archief voor de Suikerindustrie in Ned. Ind., 41: 720-721.

(9) Tanabe, T., 1937. Rept. Gov. Exp. Sta. Tainan, Formosa, 4: 33-39.

(10) Taylor, C. S., 1919. The Presence of Aconitic Acid in Sugar Cane Juice and a New Reaction for the Detection of the Acid. Jour. Chem. Soc. Transactions, 115: 886-889.

(11) Von Lippmann, 1879. Ber. Deutsch Chem. Ges., 12: 1649.

(12) Ventre, E. K., 1940. Preliminary Report of the Crystallization of Sucrose from Juices or Sorgo Plant. The Sug. Jour. No. 7, 3: 23-30.

(13) Yoder, P. A., 1911. Notes on the Determination of Acids in Sugar Cane Juice. Jour. Ind. & Eng. Chem., 3: 640-646.

(14) Zerban, F. W., 1919. The Color Changes of Sugar-Cane Juice and the Nature of Cane Tannin. Jour. Ind. & Eng. Chem., 11: 1034-1036.

Depth of Planting Cane Affects Germination

By R. J. Borden

The depth of soil that is used in covering cane seed can influence not only the germination and establishment of the "stand" of cane but also the development of the crop during its early growth stages. Furthermore the results are not always similar when different depths of planting are used with different cane varieties or in different soils.

The accompanying set of photographs, taken at two, four, and six weeks after planting single-eyed seed pieces of three cane varieties at various depths in small pots of two different soils, offer visible evidence of the germination and initial growth differences which resulted from these several differentials, and suggest that these growth variations which are established early may be greatly magnified later on if the faster-starting stalks are in a position to shade out the slower ones. The data in Tables I and II support this photographic evidence.

The principal objective of this skirmish test (A-105—No. 159) was to note the effect on germination and the average rate of spindle emergence from cane seed covered by various depths of soil. Two soils were used: (1) an alluvial clay loam of nut-like structure from Makiki, and (2) a residual silty loam with an excellent granular structure from Manoa. Three cane varieties were included: H 109, 31–1389, and 32–8560.

Prime top-seed pieces were cut from variety plantings at Makiki which were comparable in age and condition. Careful selections of comparable single-eyed cuttings were subsequently made from these seed pieces and their ends were immediately dipped in Ceresan to protect them against rapid decay. After supplying both soils with phosphate, four of these cuttings were placed in each of eight small pots and covered either one inch, three inches, or five inches; thus a total of 32 eyes were planted for each treatment. All pots of soil were then wet to their field capacities and placed on cars which were run under glass shelter at night and during heavy rains.

Daily records were made of spindle emergence and from these the percentages of germination and the true average number of days for emergence were calculated. These data are summarized in Tables I and II.

TABLE I

PER CENT GERMINATION

	MAKIKI SOIL			MANOA SOIL			Variety
Varieties	1"	3"	5"	1"	3"	5"	average
H 109	81	84	0	97	88	60	68%
31–1389	100	95	44	100	95	100	88%
32–8560	100	97	50	100	100	50	83%
Average	94%	92%	31%	99%	94%	70%	

Average for soil: Makiki = 72% Manoa = 87%

Average for depth of cover: At 1" = 96% At 3" = 93% At 5" = 51%

TABLE II

AVERAGE DAYS TO EMERGE FROM SOIL

Varieties	MAKIKI SOIL Depth of cover			MANOA SOIL Depth of cover			Variety average
	1"	3"	5"	1"	3"	5"	
H 109	11.5	17.6	....	10.6	15.8	16.8	14.5 days
31–1389	7.8	10.1	26.5	6.6	9.6	11.5	12.0 days
32–8560	10.0	12.7	19.5	8.6	11.0	18.0	13.3 days
Average	9.8	13.5	23.0	8.6	12.1	15.4	

Average for soil: Makiki = 14.4 days   Manoa = 12.0 days

Average for depth of cover: 1" = 9.2 days   3" = 12.8 days   5" = 18.5 days

Several facts seem to be quite clearly shown by these data in Tables I and II:

1. Except for the variety 31–1389 in Manoa soil, a covering of five inches of soil was responsible for a greatly reduced percentage germination. Differences between coverings of three inches and one inch were not highly significant.

2. Germination was significantly better in the Manoa soil. (Note: The Manoa soil has a more porous open structure which gives it better aeration than the Makiki soil.)

3. In all comparisons the 31–1389 seed gave a higher percentage germination than H 109 seed of comparable age and quality. Seed from 32–8560 germinated better than H 109 except when planted five inches deep in Manoa soil. Differences in the per cent germination between 31–1389 and 32–8560 were not significant except on Manoa soil with five inches of cover.

4. Depth of covering the seed had a very significant effect on the average number of days before the spindles emerged from the ground. Spindles from seed covered three inches appeared three days later than from seed planted one inch deep and when five inches of soil covered the seed, still another six days (for a total of 18 days) were required before they "broke ground." (Under wet soil conditions, rapid decay of the seed piece could take place within this average 18-day period.)

5. Seed of all varieties, regardless of depth of cover, germinated in fewer days when planted in the Manoa soil.

6. The 31–1389 shoots appeared earlier than 32–8560 except on Makiki soil when covered five inches. H 109 was slowest except on Manoa soil with five inches of cover. However, H 109 seed failed to germinate at all on Makiki soil when covered five inches.

To sum up: the percentage of seed-piece eyes which germinated and the average number of days needed for their emergence was influenced by their depth of soil covering, and five inches of cover produced undependable and usually unsatisfactory results. Thus a uniform and shallow covering should give us best results when cane is planted on either irrigated or naturally moist soils.

VARIETY H 109

On Makiki soil
Seed covered 1, 3, and 5 inches

On Manoa soil
Seed covered 1, 3, and 5 inches

1925 1937 1940   1859 1863 1870

Age - 2 Weeks

1930 1936 1941   1858 1865 1869

Age - 4 Weeks

1924 1933 1942   1852 1861 1871

Age - 6 Weeks
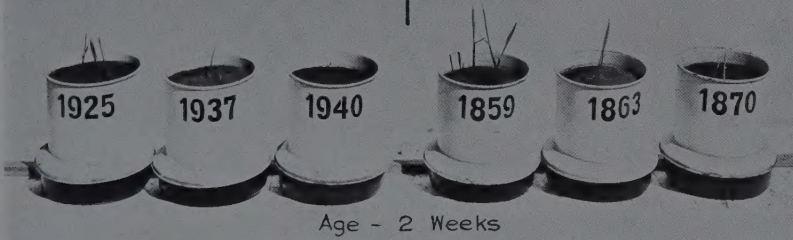
VARIETY 51-1389

On Makiki soil
Seed covered 1, 3, and 5 inches

On Manoa soil
Seed covered 1, 3, and 5 inches

1954 1956 1966   1880 1890 1896

Age - 2 Weeks

1951 1959 1969 1876 1885 1899

Age - 4 Weeks

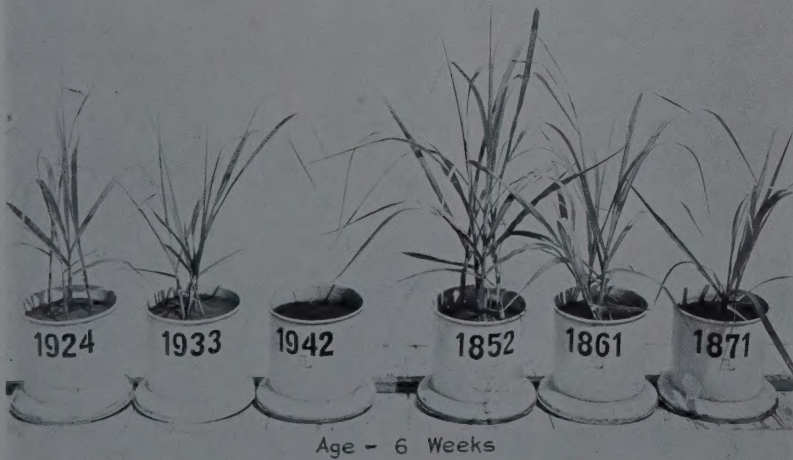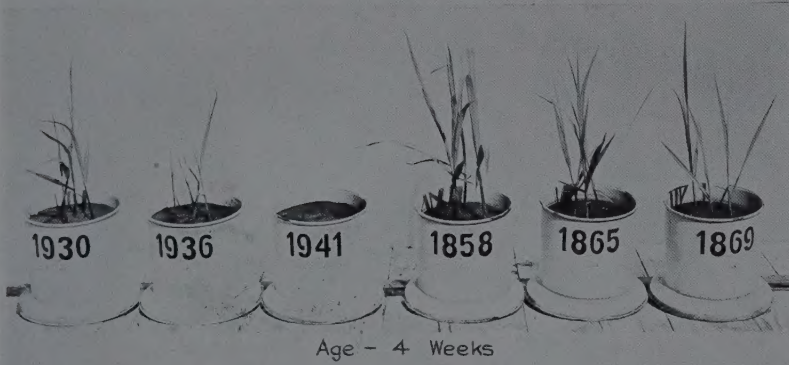1949 1958 1968 1878 1891 1893

Age - 6 Weeks
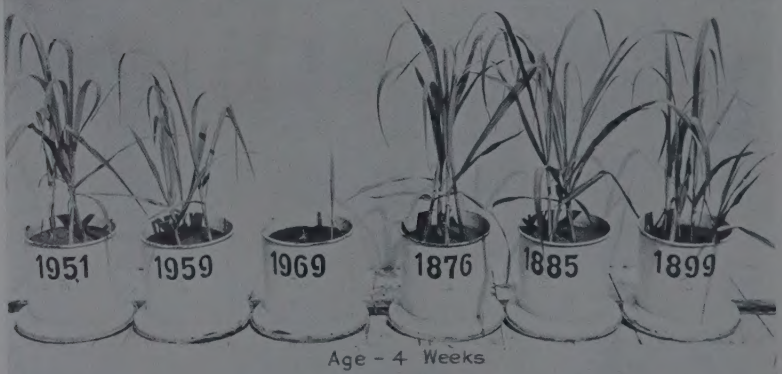
VARIETY 32-8560

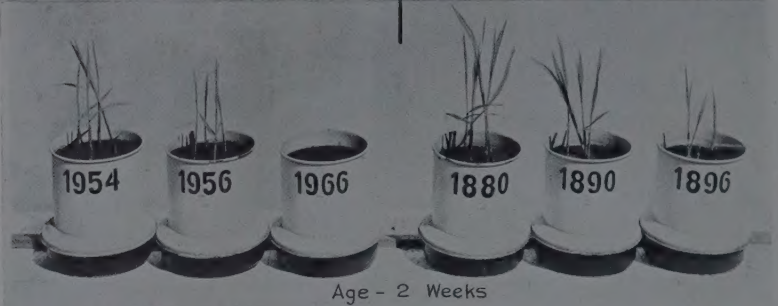On Makiki soil
Seed covered 1, 3, and 5 inches

On Manoa soil
Seed covered 1, 3, and 5 inches

1977 1984 1990 1903 1911 1916

Age - 2 Weeks

1975 1985 1995 1902 1910 1918

Age - 4 Weeks

1973 1980 1988 1900 1909 1919

Age - 6 Weeks
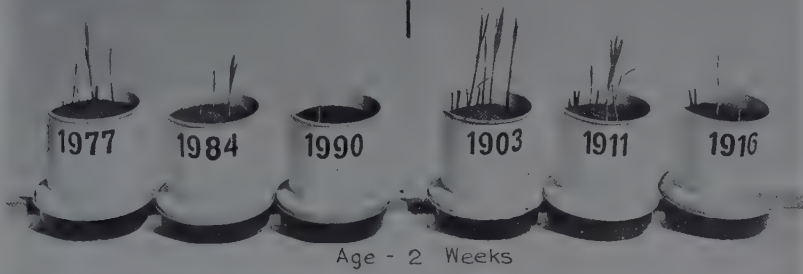
A Recent Immigrant Tachinid Fly Parasite of
Noctuid Caterpillars in Hawaii

By R. H. Van Zwaluwenburg

The presence is noted of a recently arrived fly on Oahu which parasitizes a number of different kinds of destructive caterpillars, among them the corn-ear worm. In the laboratory it bred readily on the nutgrass armyworm also. The fly has a wide range in continental North America and in the West Indies, and evidently arrived here by chance within wormy tomatoes imported from Mexico. Its introduction was unsuccessfully attempted in 1923. The fly has an unusually brief life cycle, and the female, instead of laying eggs, deposits nearly mature maggots within the body of its victim.

In April 1942 a fly, new to the Islands and belonging to the family Tachinidae, was seen for the first time in the field on Oahu. C. E. Pemberton and the writer noticed considerable numbers of what were at first taken to be somewhat over-sized individuals of the sugar cane beetle borer parasite, in a potato field adjacent to cane at Waialua Agricultural Company. Closer examination of the specimens showed them to be identical with a specimen bred in Honolulu by Dr. F. X. Williams in March 1941 from a caterpillar in a tomato, probably from Mexico, purchased in the local market. Dr. Williams identified it as *Eucelatoria armigera* (Coquillett). Additional specimens were later found among unidentified material collected for introduction into Hawaii by H. T. Osborn from the state of Vera Cruz, Mexico, in 1923. Osborn's shipments were all dead upon arrival in Honolulu, due to the very short life cycle of the fly. It is presumed that the fly became established through the arrival of individuals in imported tomatoes, like the specimen which Dr. Williams intercepted. Incidentally this is a good example of how immigrant insects succeed in arriving here from distant places. Had *Eucelatoria* been an undesirable insect it would have been a successful evasion of plant quarantine precautions; that the species happens to be a welcome addition to the fauna is fortunate. It is not known, of course, how long this fly has been established here, but from its abundance when first found in the field, it may well have been several years. As suggested above, it somewhat resembles the *Ceromasia* parasite of the sugar cane beetle borer (see Fig. 1) ; however, it is slightly larger, measuring from about 6 to 8 mm., or ¼ to ⅓ inch in length.

E. armigera was first described by Coquillett in 1889 (4, p. 332) from material bred from the corn-ear worm, *Heliothis armigera* (Hübner), in Los Angeles, California, under the name *Tachina (Masicera) armigera*. It has since been variously placed in the genera *Lydella, Blondelia, Anetia* and *Frontina*. Townsend in 1909 (8, p. 249) assigned it to a new genus, *Eucelatoria*.

Besides the corn-ear worm, this fly has several other hosts, mainly among the moth family Noctuidae. Osborn (6, p. 150) found *Cirphis latiuscula* (Herr.-Schff.) so heavily parasitized by what later proved to be this fly at El Potrero, Vera Cruz in July and August, that very few reached the adult stage. He says: "From the rapidity with which it overtook a threatened outbreak of the *Cirphis* it seemed

Fig. 1. Dorsal view of adult male *Eucelatoria armigera* (Coq.). (X12) (Drawn by J. T. Yamamoto.)

to me as possibly the most promising of the parasites observed on the armyworm in Mexico." Blanchard and Conger (2, p. 1065) bred this fly in California from larvae of *Prodenia praefica* Grote, an important pest of alfalfa and other field crops. Bruner (3, p. 96), crediting Cardin, records rearing *E. armigera* from *Laphygma frugiperda* (A. & S.) in Cuba. In May 1942 E. C. Zimmerman first reared it from the garden looper, *Plusia chalcites* (Esp.), feeding on foliage of *Spathoglottis* orchids in Honolulu. Subsequently it was found to parasitize this insect commonly; O. H. Swezey found that of 41 *Plusia* caterpillars collected at Ewa in December, about 36 per cent were parasitized by it. In the laboratory we have reared it without difficulty from half- to full-grown larvae of *Laphygma exempta* (Walker), the nutgrass armyworm, as well as from *Heliothis*. Attempts to induce parasitism of *Pieris rapae* (Linn.), cabbage butterfly larvae, were unsuccessful.

Greene's record (5, p. 43) of *E. armigera* bred from a sawfly larva, *Neodiprion sp.* in Georgia, shows that this fly does not entirely confine itself to moth caterpillars. Sawflies are members of the hymenopterous family Tenthredinidae; they are not represented in Hawaii. Wilson (11, p. 13) records a related fly, *Eucelatoria rubentris* (Coq.), parasitic in *Laphygma exigua* (Hübner) in Florida.

Because *E. armigera* has such a number of hosts among the generally destructive noctuids, it gives promise of considerable value under Hawaiian conditions. Should it take as readily to *Laphygma* in the open as it does in the laboratory, much may be

expected from it in control of the nutgrass armyworm. The fact that it attacks a species of *Prodenia* in California makes it reasonably certain that if *Prodenia litura* (Fab.), a general pest of major importance in the Pacific, should ever become established here, we shall have on hand a parasite to combat it.

E. armigera has the following wide range: California, Georgia, Florida, Texas (1, p. 943) and eastern Mexico on the mainland of North America, and Cuba and Puerto Rico (12, p. 353) in the West Indies. So far its establishment in Hawaii is certain only on Oahu, but small colonies have been sent to central Maui where the fly should become established with little difficulty.

Instead of laying eggs as most flies do, the female *Eucelatoria* deposits nearly mature larvae, or maggots, within the body of its victim, piercing the body with a highly specialized larvipositor (see Fig. 2). This mechanism (the "sternotheca" of Townsend) is a heavily chitinized, curved, thorn-like organ, widened toward the

Fig. 2. Lateral view of larvipositor of female *E. armigera;* only a small portion of the lower curve (below a line drawn from A to B) is normally visible. For this illustration all the concealing structures have been removed. (Drawn by J. T. Yamamoto.)

base, and flattened and channelled on its outer curve. A small flap, or valve, overlies the channelled portion at the base, and the maggots are extruded from between this valve and the main part of the larvipositor. The process of piercing the integument of the caterpillar and depositing the maggot within takes place with extraordinary swiftness, and consists of a "flashlike dart at the host. The fly no sooner makes this dart than she is away again, yet during this brief fraction of a second the act of subcutaneous larviposition has been accomplished." (9, p. 90). When through feeding within the host, the maggot leaves the body of its victim and enters the soil to form a puparium, or pupal case. Caterpillars that have been pierced can usually be identified by the dried discoloration of the body fluids issuing from the puncture. Parasitized *Laphygma* larvae suffer complete collapse, sometimes within a day of being stung. *Heliothis* and *Plusia,* however, continue active and apparently normal for sometimes as much as five days after attack.

Townsend (7, p. 117) lists five different methods of reproduction among flies of this family: (1) host-oviposition; (2) leaf-oviposition; (3) supracutaneous host-larviposition; (4) subcutaneous host-larviposition; and (5) leaf-larviposition. With the advent of *Eucelatoria* we now have, among the seven tachinid flies in Hawaii, examples of four of these five methods, as well as still another in the case of *Ceromasia:*

Frontina archippivora Williston	Eggs laid on host
Leucostoma aterrima Williston	Eggs laid within host (coreid bugs of the genus
Leucostoma atra Townsend	*Corizus*)
Chaetogaedia monticola (Bigot)	Eggs laid on foliage, to be ingested by the host
Eucelatoria armigera (Coquillett)	Maggots deposited within host
Archytas cirphis Curran	Maggots deposited on foliage frequented by host
Ceromasia sphenophori (Villeneuve)	Maggots deposited loosely within tunnel of sugar cane beetle borer

The life cycle of *Eucelatoria* is very short, much shorter than that of any of the other species listed above, with the possible exception of *Leucostoma* on which little information is available. Even during the winter months *Eucelatoria* can complete a generation in less than three weeks. From larviposition to the emergence of flies of the next generation took from 12 to 13 days during January and February. Females were able to larviposit six days after emergence. Thus even in winter the entire life cycle takes from 18 to 19 days; during warmer parts of the year this may be expected to be somewhat reduced. Newly emerged flies mated within eight hours or less.

From 176 rearings of laboratory-bred *Eucelatoria* it appears that the proportion of sexes is very nearly equal: 86 males and 90 females.

	Reared Jan. 20–Feb. 7 Avg. mean temp. 72.9° F.		Reared Feb. 11–Mar. 7 Avg. mean temp. 69.1° F.	
	39 males	40 females	47 males	50 females
Days from larviposition to emergence of adult fly	12.0	12.2	12.9	13.2
Days spent by maggot within caterpillar	4.3	4.0	4.2	4.0
Days in puparium	7.6	8.2	8.6	9.2

Females required a slightly longer pupal period than males did. Males spent a somewhat longer time within the host than females; this feeding period was about the same irrespective of temperatures outside the host. However, the pupal period was prolonged by cooler temperatures, with a corresponding lengthening of the total period from larviposition to emergence.

The largest number of flies developing from a single caterpillar under field conditions was nine in the case of a *Plusia* larva. This number varies with the size of the host, and perhaps also with the number of flies present in the field and the number of caterpillars available. Concerning *Eucelatoria* parasitizing *Cirphis* in Mexico, Osborn (6, p. 150) says: ". . . several may develop in a single host. Occasionally up to five or six may be obtained, although many caterpillars have only one, and the average [is] probably not over three or four." If a larva is exposed to too many flies, super-parasitism may result, that is, more maggots (14 in the case of one small *Plusia* larva) are deposited than can develop. The results of breeding with *Laphygma* larvae in the laboratory were as follows:

31 caterpillars (Jan. 20–Feb. 7) produced 39 males and 40 females, or 2.5 flies per larva.
34 caterpillars (Feb. 11–Mar. 7) produced 109 puparia (3.2 per larva) from which issued 47 males and 50 females, or 2.8 flies per larva.*

As a rule the more flies developed per larva the smaller their size, although the size of the host also is a factor, of course. Flies from a five-puparia lot bred from a

* To make observation easier, no soil was present in the containers in which the maggots pupated; had puparia been formed under more nearly natural conditions the percentage of successful emergences might have been higher.

last-stage corn-ear worm were larger than those from a lot of equal number reared from a last-stage nutgrass armyworm. Of 32 *Laphygma* larvae:

> 7 produced 1 fly puparium each
> 8 produced 2 fly puparia each
> 6 produced 3 fly puparia each
> 1 produced 4 fly puparia each
> 8 produced 5 fly puparia each
> 2 produced 8 fly puparia each

L. R. Smith of our Agricultural department has examined the following data and found them to have statistical significance. They show an inverse ratio between the number of *Eucelatoria* maggots in a single larva, and the length of time spent by the maggots within the host:

No. of flies produced per host larva	Days spent within host larva
1 (7 examples)	5.4
2 (8 ")	5.0
3 (6 ")	5.0
4 (1 ")	4.0
5 (8 ")	3.5
8 (2 ")	3.2

Nearly mature maggots were several times observed to extrude the caudal end of the body through a break in the skin of the host. After some minutes thus exposed they would retreat into the host again. The maneuver suggests a method of respiration, the respiratory spiracles being situated on the posterior end of the maggot's body.

Under laboratory conditions, fed on brown sugar and water, the maximum length of life of male *Eucelatoria* was between 17 and 18 days. Reproductive females lived a maximum of from 17 to 18 days, while one female, presumably mated, but sterile, lived for 30 days.

The maximum productiveness noted was 42 puparia each in the case of two females; the potential productiveness is certainly much higher. According to Townsend (10, p. 49) *Eucelatoria* has a capacity of 100 to 200 maggots. Of 11 females confined with males and having almost daily access to caterpillars for larviposition, only three reproduced in the laboratory:

Female B— lived from 17 to 18 days; when 12 days old parasitized a *Laphygma* larva, the only one successfully parasitized by this female; one puparium and a male fly resulted.

Female C—lived from 15 to 16 days; first parasitized two of three *Laphygma* offered, when six days old; parasitized ten *Laphygma* and one *Heliothis* on nine different days, the last when the fly was 14 days old; five *Laphygma* offered it were not attacked. This fly produced 42 puparia from which 16 males and 20 females issued.

Female E—lived from 13 to 14 days; first parasitized two of three *Laphygma* offered, when six days old; parasitized one *Heliothis* and nine *Laphygma* on seven different days, the last when the fly was 12 days old; two *Laphygma* offered it were not attacked. This individual produced 42 puparia from which 19 males and 19 females issued.

Several of the apparently sterile females stung *Laphygma* larvae so severely that they soon showed the collapse typical of parasitized individuals, but no flies resulted.

Fig. 3. Dorsal view of head of *E. armigera* (male, left; female, right) showing comparative width of the interocular space and differences in setation. (Drawn by J. T. Yamamoto.)
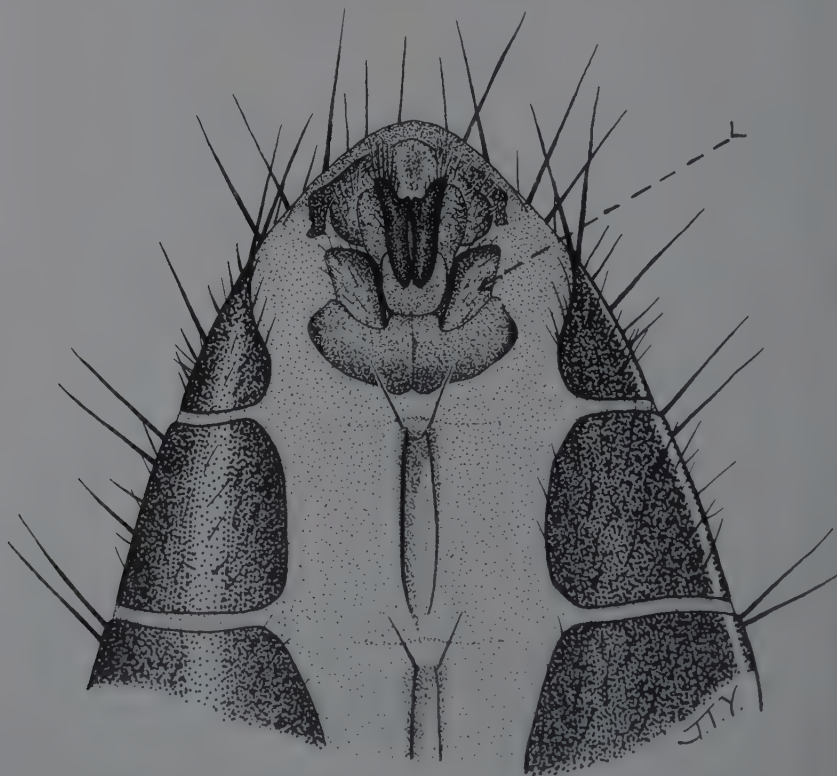

Fig. 4. Ventral view of abdomen of male *E. armigera*. In this drawing the plates concealing the male hypopygium are purposely separated to show the fifth sternite with its lobes (L) which distinguish the male from the female. Normally only the tips of these lobes are visible. (Drawn by J. T. Yamamoto.)

As early as the fifth day after emergence female flies showed interest in *Laphygma,* and a few flies of that age attacked larvae. It is doubtful if maggots were actually deposited by five-day-old females; no reproduction was obtained in such instances.

Townsend (10, p. 49) states that the uterus of *Eucelatoria* is "in 3 or 4 coils or loops with the eggs and maggots obliquely on end in single or double file. . . ." Examination of fresh material showed the eggs at the upper end of the tubes to be obliquely placed at about a 45-degree angle; as they progress downward the eggs develop into maggots, which gradually assume a longitudinal position with the caudal end downward. The eggs as described by Townsend (9, p. 88) are "stout subcylindric, rather elongate, slightly tapered at ends, chorion membranous and transparent." The maggots are "stout subcylindric, white, with more or less complete transverse spine rows, the spines more numerous on ventral surface and the fourth segment more extensively spined than the others, the last segment tipped dorsally with 3 short, stout, hooked spines."

The sex of *Eucelatoria* flies can be distinguished with little difficulty: (1) the space between the eyes (see Fig. 3) is relatively wider in the female than in the male, and the setae, or hairs, on the upper part of the front are arranged in a double row in the female, in a single row in the male; (2) the intermediate segments of the underside of the female have, along the middle line, a strongly spined carina, or ridge; this is absent in the male, which has two more or less rounded, readily visible lobes on the posterior margin of the fifth ventral segment (see Fig. 4).

LITERATURE CITED

(1) Bibby, F. F., 1942. Some parasites of Heliothis armigera (Hbn.) in Texas. Journ. Econ. Ent., 35: 943–944.

(2) Blanchard, R. A., and Conger, C. B., 1932. Notes on Prodenia praefica Grote. Journ. Econ. Ent., 25: 1059–1070.

(3) Bruner, S. C., 1934. La Palomilla del maiz. Revista de Agr. (Cuba), 14: 94–97.

(4) Coquillett, D. W., 1889. The corn worm or boll worm in California. Insect Life, 1: 331–332.

(5) Greene, C. T., 1921. Dipterous parasites of sawflies. Proc. Ent. Soc. of Washington, 23: 41–43.

(6) Osborn, H. T., 1938. The introduction into Hawaii from Mexico of insect parasites to control armyworms (1923–1924). The Hawaiian Planters' Record, 42: 147–152.

(7) Townsend, C. H. T., 1908. A record of results from rearing and dissections of Tachinidae. U. S. Dept. Agr. Bur. of Ent., Bull. Tech. Ser. 12, pt. 6, 95–118.

(8) ————, 1909. Descriptions of some new Tachinidae. Ann. Ent. Soc. of America, 2: 243–250.

(9) ————, 1936. Manual of myiology, pt. 4, 309 pp.

(10) ————, 1940. Manual of myiology, pt. 10, 334 pp.

(11) Wilson, J. W., 1933. The biology of parasites and predators of Laphygma exigua Huebner reared during the season of 1932. Florida Ent., 17: 1–15.

(12) Wolcott, G. N., 1936. Insectae Borinquenses. Journ. of Agr., Univ. of Puerto Rico, 627 pp.

The Production of Sorgo Sirup and Sugar

By H. A. Cook

Sorgo cane is considered to possess very desirable agronomical characteristics; it matures very quickly and can be grown over wide areas in the United States. Crystallization of sugar from sorgo juices has been a very difficult problem and much research has been conducted toward that end for many years. During recent years research carried on largely under the guidance and cooperation of the Carbohydrate Unit of the Agricultural Chemistry Division, Bureau of Agricultural Chemistry and Engineering, United States Department of Agriculture, has been responsible for the development of a process whereby the crystallization of sucrose has been effected. This has revived considerable interest in the sorgo plant on the mainland. A review of this work is presented.

During recent years interest in the production of sorgo sirup and the manufacture of sugar from sorgo juices has been revived on the mainland. Under present conditions it appears that this interest may become more active; we therefore present an outline of some of the developments which have taken place, especially in regard to the crystallization of sucrose (cane sugar) from sorgo juices. The research was carried on largely under the guidance and cooperation of the Carbohydrate Unit of the Agricultural Chemistry Division, Bureau of Agricultural Chemistry and Engineering, United States Department of Agriculture.

Prior to 1933, reports from the U. S. Department of Agriculture refer to the poor or non-uniform quality of sorgo sirup produced on many farms in the United States. A recent report by E. K. Ventre of the above bureau summarizes the early situation in The Sugar Journal, Vol. 3, December 1940, p. 23, as follows:

The crystallization of sucrose from the juices of sorgo plant is a project that has had much intensive study in the past. The previous investigators succeeded in demonstrating the facts that the plant has very desirable agronomical characteristics and sucrose content, which compared very favorably with those of sugarcane and sugarbeets at that time. However, due to the failure to develop a workable process for the extraction of sucrose from the juices the investigations were abandoned.

In a similar report made to the Chief of the Bureau of Agricultural Chemistry and Engineering, U.S.D.A., 1940, pp. 28–29, is the following statement:

Utilization of suitable varieties of the sorgo plant for the production of sugar and its byproducts is a possibility that may be of great importance under some circumstances, and renewed attention has been given to this problem. It now appears that the difficulties which previously handicapped this development may be overcome. Sorgo matures in about 4 months and can be grown over wide areas in the United States. In certain sections it could be used to supplement sugarcane and sugarbeets, making possible a longer and more profitable period of operation of existing cane- and beet-sugar factories and providing a new and profitable crop to growers.

The lack of uniformity in the quality of sirup, sedimentation occurring in the sirup, and the inability to precipitate sucrose from the juices was a real problem in the sorgo-producing areas of the United States. The report of the Chief of the Bureau of Chemistry and Soils of the Department of Agriculture, 1935, p. 7, summarized the situation as follows:

This defect has been a serious handicap to farmers in obtaining the fullest profit from this important cash crop. Investigations were continued for the purpose of devising means whereby sorgo sirup of more uniform and improved quality may be made directly on the farm.

A continuation of the report states:

One of the principal difficulties experienced by sorgo sirup producers is slow boiling, which often results in scorching the sirup. This trouble was found to be caused by the presence of starch in the juice, which in addition to retarding evaporation will, if present in large quantity, cause actual jellying of the sirup.

A practical method was devised for use of malt extract in the production of sorgo sirup for the elimination of defects such as ''jellying,'' excess turbidity, and difficulty in concentrating the sirup to the required density, which result from the gelatinization of starch contained in the juice. This solves a difficulty which has in the past been the cause of much poor-quality sorgo sirup.

The report continues:

The use of a high diastatic malt for hydrolyzing the starch is recommended to overcome this difficulty. It was found that the greatest benefit from the use of malt is obtained by applying it after the juice has been evaporated to a semisirup. The usefulness of the diastase method for preventing slow boiling, scorching, and jellying of sorgo sirup was demonstrated in cooperative work with the Arkansas Agricultural Experiment Station. The drought of 1934 apparently was responsible for the fact that it was practically impossible to produce sirup of satisfactory quality by the usual farm methods. By employing the newly developed improved method, sirup of excellent quality was produced from the same sorgo cane.

Work on this subject was continued in 1936 along with studies relating to the factors of ripeness of the cane and topping the cane. This is summarized in the Report of the Chief of the Bureau of Chemistry and Soils, U.S.D.A., 1936, p. 5.

In an effort to eliminate the difficulties which tend to prevent the production of good sorgo sirup, an investigation was made of the quality and composition of sirups prepared from different portions of the sorgo stalk at different stages of maturity. This work was done in cooperation with the Mississippi Agricultural Experiment Station, and four of the best varieties of sorgo grown in northern Mississippi were studied. The data obtained show that the quality of sorgo sirup can be greatly improved by using cane which is ripe but not overripe, as determined by the condition of the seed heads, and also by discarding a certain proportion of the cane tops. The improvement in quality and possible increase in value may offset the comparatively small loss in yield of sirup, particularly when the forage value of the tops is considered. Most of the starch, which causes ''jellying'' and makes it impossible to boil down the sirup to required density without scorching, is present in the upper part of the stalk and can be eliminated by topping. A study of the sucrose and reducing sugar relationship in different parts of the stalk also revealed that it might be practical to establish a practice of selecting a certain portion of the sorgo stalk for sirup production, whereby sucrose and dextrose crystallization in the sirup, which detracts from its value, could be avoided without incurring too great loss in yield of sirup. Objectionable sharp flavor or ''tang,'' which is correlated with the titratable acidity, can probably be minimized by variety selection, avoiding overripe stage of maturity, and topping to the fifth internode.

Studies in cooperation with the Mississippi Agricultural Experiment Station continued and were incorporated in the Report of the Chief of the Bureau of Chemistry and Soils, 1937, p. 5.

The malt diastase process for the prevention of jellying and consequent slow boiling or scorching and the practice of topping the stalks to the fifth internode to reduce the mineral, acid, and starch content of the juice fully meet the limitations of farm-scale sirup production. Studies conducted ... during the year showed that further improvement in quality of sirup could be accomplished by combining these two methods.

Cooperative work in Mississippi on the composition of juices and quality of sirup from different sections of the stalks, in which 8 additional varieties of sorgo cane were used and about 100 samples of sirup were made, confirmed the conclusions from last year's work that the farm value and marketability of sorgo sirup can be materially improved by cutting off and discarding several joints of the stalk from the top. The discarded top joints can be well utilized as feed for livestock, and the comparatively small reduction in yield of sirup is more than compensated by the improvement in quality of the sirup and the feeding value of the discarded top sections of the stalks.

Farmers' Bulletin 1791, *Farm Production of Sorgo Sirup,* by C. F. Walton, Jr., E. K. Ventre and S. Byall, was published in 1938 and contains a very complete treatise on the manufacture of sorgo sirup on a small farm scale. This bulletin is based upon and embodies the results of the preceding work on this subject. It covers the topping and milling of the cane; location, layout and size of mill and equipment; treating and evaporating the juice; removing sediment from the sirup; treatment with diastase; how to prevent sugaring; problems and methods of canning, marketing, etc. The juice is extracted with a small two- or three-roller mill, passing the cane through twice, and obtaining an extraction of 55 to 68 per cent. This juice may be centrifuged to remove a large amount of the starch, or merely neutralized with lime and heated. After clarification it is boiled to a semisirup. The semisirup is treated with diastase malt to hydrolyze the starch, and settled for a period of six hours to remove sediment. They state (p. 34) that dextrose crystallization is controllable by blending of varieties of cane or of sirup:

Success in preventing crystallization of both dextrose and cane sugar nevertheless depends upon having these sugars present in the sirup in the right proportions.

The proportions usually satisfactory are about equal amounts. They suggest also (p. 34) the use of invertase:

Another method of preventing cane-sugar crystallization, if variety selection, harvesting at the proper stage of maturity, and low topping fail to give good results, is the "invertase process." This is a practicable process in which an extract of yeast (invertase) is used during the manufacture of the sirup. The yeast extract converts a portion of the cane sugar into the two sugars dextrose and levulose, so that the resulting more properly balanced sugar content of the sirup will not deposit either cane sugar or dextrose crystals. The invertase process, of course, should not be used when the trouble to be remedied is due to dextrose.

It is disclosed by these comments that difficulties were encountered through crystallization occurring in the sirup due to three forms of sugar, *i.e.,* sucrose, dextrose and levulose. These crystallize in the sirup under different conditions, depending somewhat upon their proportions in the sirup.

The subject of topping the stalks of the sorgo plant received further study in connection with the crystallization of sucrose and dextrose in sorgo sirups. E. K. Ventre and S. Byall further reported in *Distribution and Variation with Maturity of Dissolved Solids, Sucrose, and Titratable Acidity in the Sorgo Stalk,* Journal of Agricultural Research (1937), Vol. 55, pp. 553–562. They state on page 562:

If sorgo juices are to be used for the crystallization of sucrose, previously recommended topping practices are incorrect. The reverse procedure should be used, that is, the bottom internodes should be discarded, as they have relatively a much lower coefficient of purity. In some cases several internodes at the bottom of the stalk are much below the practical crystallization limits for sucrose.

In the manufacture of sirup from the sorgo stalk, however, topping, or discarding the three or four upper internodes, reduces the tendency of the sirup to crystallize sucrose and also produces sirup with a minimum of acidity or sharp "tang."

The above report makes the first reference encountered since 1933 concerning the commercial possibilities of sucrose crystallization from the sorgo sirup.

Correlation of starch content, jellying, crystallization, topping and maturity of the sorgo stalk were further studied and reported upon by E. K. Ventre, S. Byall, and C. F. Walton in 1939, *Jellying and Crystallization of Sirups Made from Different Parts of the Sorgo Stalk at Different Stages of Maturity*, Journal of Agricultural Research (1939), Vol. 59, pp. 139–150, and were summarized on page 149 as follows:

The starch content and jellying of sorgo sirups are correlated and increase with maturity of the sorgo. The upper portions of the stalk produce sirups higher in starch content. The number of parts of the stalk yielding sirups that jelly increases with maturity.

Sucrose crystallization occurs most frequently in sirups made from the upper part of the sorgo stalk. The number of parts of the sorgo stalk yielding sirups from which sucrose crystallizes increases with maturity.

Dextrose crystallization occurs most frequently in sirups made from the lower portions of the stalk. The number of portions of the stalk yielding sirups from which dextrose crystallizes decreases with maturity.

We have three reports along the same line on the same subject during 1940 One of these, also by C. F. Walton, E. K. Ventre and S. Byall, *Effects of Low Topping and Diastatic Malt Extract on Composition and Quality of Sorgo Sirup* Journal of Agricultural Research, Vol. 60 (1940), pp. 427–432, gives the following summary on page 432:

Most of the sirups with a starch content of 1.25 percent or higher, jellied or became extremely viscous, whereas those made by the use of high-diastatic malt extract had a starch content considerably under 1.0 percent, in many cases only about 0.25 percent, and did not jelly.

The ash content of the samples varied considerably with the variety but it was usually higher in sirups made from the top portion of the stalk.

In making sirups with juice from the tops alone, it was observed that scorching usually occurred before evaporation to the standard density of sirup was completed. This is characteristic of juices of high starch content, and accounts for the dark color and strong flavor of some sirups.

The topped stalks consistently produced sirup of better quality than the whole stalks, which, in turn, gave better sirup than did the tops alone.

Relatively little improvement in quality resulted from simply allowing the semisirup to settle, without malt-extract treatment, before completing the evaporation.

All sirups produced by the process in which high-diastatic malt extract was used were of better color, flavor, and clarity than the sirups made from corresponding parts of the stalk by the standard procedure.

The results show that sirups of the highest quality are produced by using starch-hydrolyzing enzymes to supplement reasonably good topping practice.

The report of the Chief of the Bureau of Agricultural Chemistry and Engineering, 1940, pp. 28–29, mentions the fact that the work had progressed so far that the primary *crystallization of sucrose could be readily accomplished*. Excerpts from the report follow:

Analyses were also made of the objectionable sediment formed to different extents in samples of sirup from various sources, in order to obtain information on the nature and origin of this sediment which may lead to the adoption of preventive or remedial measures. Farm made sirups free from sediment would have improved marketing possibilities.

Preliminary results obtained in experiments on a pilot-plant scale at Meridian, Miss., field station showed that starch and its degradation products in sorgo juices are among the most objectionable constituents that prevent efficient crystallization of sugar. Means for the re-

moval of interfering starch and starch products by physical methods and by diastatic conversion were devised, with the result that primary crystallization of sucrose could be readily accomplished. Satisfactory analytical methods were developed for determining sucrose, dextrose, and levulose in sorgo juices. The results of a study of the diurnal variations in the sucrose, dextrose, and levulose contents of the sorgo stalk were not indicative that the synthesis of sucrose and starch occurs in the stalk juice. Promising results, from the standpoint of quality, were obtained in laboratory studies on the production of refined sugar in the form of sirup (''liquid sugar'') from sorgo juice by the use of ''carbonaceous'' ion-exchange materials. Best results were obtained when the juices were passed through the cation and anion exchange materials alternately. Excessive wash water, however, was required to remove color after regeneration of the anion exchange material which is a serious objection. An investigation was begun on methods for the preparation of an ion-exchange material from fibrous materials impregnated with analine dyes.

Some interesting developments are brought out in the above report, including the commercial possibilities in the crystallization of sucrose (cane sugar) through the development of efficient means for removal of starch and its deleterious products; that materials which are ordinarily contained in the sediment of the semi-sirup may be the cause of considerable interference to free boiling in the pans and subsequent centrifugal separation; appreciable further benefits may be derived from carbonaceous ion-exchangers in further clarification of the juice, together with studies toward making fibrous ion-exchangers impregnated with analine dyes.

E. K. Ventre also described the work and the results obtained in a pilot-scale plant toward the crystallization of sucrose from the sorgo juices in *Preliminary Report of the Crystallization of Sucrose from Juices of Sorgo Plant,* The Sugar Journal, Vol. 3, No. 7 (1940), pp. 23–30, excerpts from which follow:

In the present investigations, it was found that three constituents of the juices besides the sucrose content were directly concerned with its crystallization, *viz.*, starch, reducing sugars and salts of organic acids, each of which can best be discussed separately.

Starch Content of the Juices—Influence and Methods of Removal:

[There is shown] a wide variation in the starch content of the juices; these variations are due mainly to varietal characteristics but in some instances are, in part, due to the degree of maturity of the cane. It has been shown in a previous work that the starch content and sucrose content of the juices increase with maturity. The juices of the plant contain mature starch and intermediates from various stages in the process of synthesis which do not lend themselves to physical separation and require the use of enzymes for their conversion.

Physical Separation of Starch:

The first method employed for physical separation of the starch was liming the juices to neutrality, heating to boiling point in an open defecator, thereby raising the starch to the top of the defecator and drawing off the clear juice from under the ''blanket'' of scums. Reference ... will show that this method is effective to the extent of removing an average of 70.92% of the starch originally present in the juice.

The second method studied for the physical removal of starch from the juice was by tabling the raw juice. ... When followed by lime and heat defecation we find ... an average removal of 80.80% of the starch originally present in the juice. The tabling process had the disadvantages of requiring a large installation due to a low tabling rate and permitted deterioration of the juices besides a considerable juice loss when the tables were drained.

The third method studied for the physical removal of starch from the juice was centrifuging in a solid basket centrifuge. ... this method alone removed an average 70.72% of the starch originally present in the juice and in combination with defecation removed 92.57% of the starch originally present in the juice. This method possessed, in addition, the advantages of no deterioration hazard due to the rapid separation, and the starch removed was in a compact form more suitable for use as a by-product. Varying with the variety, it is shown ... that from 0.3 to 9.85 pounds of starch per ton of cane may be recovered as a by-product by this method.

Enzyme Treatment for Removal of Residual Starch and Intermediates:

The influence of starch on the crystallization of the sirups under vacuum is first noted by the sirups becoming gelatinous and not circulating at a concentration below that necessary for securing the degree of supersaturation required for crystal growth. This does not involve a large amount of starch; for example, at 88% solids only 12 percent of water is present and in sirups having as much as .40% starch on solids the starch percent water would be 3.5% sufficient to cause a jell at this concentration. Further concentration of the starch obtains in the molasses and from this it is readily evident that nearly complete starch removal is neces-sary for free working of the juices in the factory. ... it was found necessary subsequent to physical separation to use enzymes for the final removal of starch and its products. The first enzyme used was an infusion of ground malted barley. The use of this material as an enzyme source was successful insofar as securing crystallization but it possessed the disadvantages of

PROCESS USED FOR SORGO PILOT PLANT OPERATION

The process that gave the most satisfactory operation may be diagramed as follows:
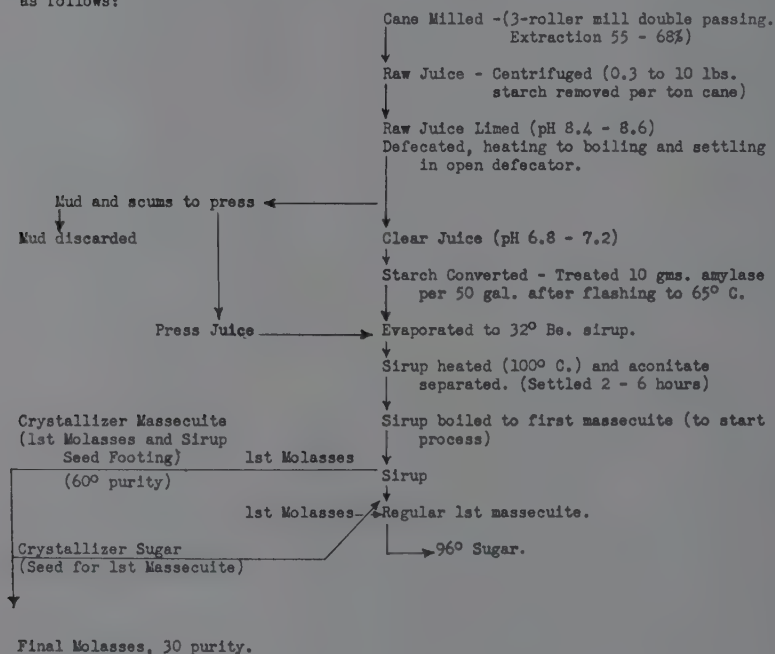
Cane Milled -(3-roller mill double passing. Extraction 55 - 68%)

Raw Juice - Centrifuged (0.3 to 10 lbs. starch removed per ton cane)

Raw Juice Limed (pH 8.4 - 8.6)
Defecated, heating to boiling and settling in open defecator.

Mud and scums to press ←

Mud discarded

Clear Juice (pH 6.8 - 7.2)

Starch Converted - Treated 10 gms. amylase per 50 gal. after flashing to 65° C.

Press Juice ⟶ Evaporated to 32° Be. sirup.

Sirup heated (100° C.) and aconitate separated. (Settled 2 - 6 hours)

Crystallizer Massecuite
(1st Molasses and Sirup
    Seed Footing)   1st Molasses
    (60° purity)

Sirup boiled to first massecuite (to start process)

Sirup

1st Molasses—⟶ Regular 1st massecuite.

Crystallizer Sugar
(Seed for 1st Massecuite)

⟶ 96° Sugar.

Final Molasses, 30 purity.

requiring an amount of infusion which added a considerable amount of reducing sugars to the sirup, required a pH below that optimum for the clarification and gave a low conversion to monosaccharides. However, this is one of the cheapest sources of starch-converting enzymes and due to this fact this material will be the subject of further study.

Pancreatic amylase was found to possess the advantages of working at the pH of optimum clarification, gave a high conversion to monosaccharides, and added no reducing sugars of itself to the sirup. In the use of either enzyme it was found most convenient to draw the hot defecated juice into a vacuum evaporator flash to cool to 65° C. and add the enzyme being careful to maintain a temperature below 70° C. during the time required for the enzyme to convert the starch. It was found that for sirup so prepared to boil satisfactorily that it show neither starch nor dextrin test with iodine. Using these methods and present equipment it was found that it required 40 grams per ton of cane of this material to get a working sirup. Further work on this should permit a reduction in the enzyme demand.

Calcium Salts or Organic Acids [*Salts of Organic Acids—Aconitates*]:

Starch free sirups prepared by a combination of the physical and enzymatic methods were found to be difficult to work for the reason that when they were heated and concentrated in the vacuum pan crystals formed at a concentration below the degree of supersaturation necessary for sucrose crystallization. These crystals continued to grow until they reached a ''smear'' size and then stopped growing. When sucrose crystals were subsequently formed, these crystals remained as a ''smear.'' They appeared to be lighter in density than the sucrose crystals as they came to the center of the centrifuge forming an impervious film preventing the massecuite from spinning.

It was found that if the evaporator sirup was heated to 100° C. these crystals formed and readily settled to the bottom. After six to ten hours' time, as much as 10 pounds of this material per ton of cane could be separated of the consistency of wet sand. Analysis of this material indicated that it was mainly calcium aconitate with a small amount of magnesium aconitate. It was found possible to further remove only a small amount of this material from first and second molasses made from sirups previously treated.

[It was shown] that the ash removal for sirup treated by heat and settling from six varieties to be a nearly constant figure of 0.72% ash on solids. Calculations based on these figures show an average ash removal of 1.545 pounds per ton of cane.

On the subject of *Reducing Sugars and Their Effect on Crystallization,* a tentative formula is presented for calculating the amount of crystallization of sucrose in relation to invert sugars.

It is interesting to note that thirty purity molasses may be produced up to a reducing sugar (as invert sugar) content percent solids of 13.20%.

Again it is desirable to emphasize that this paper must be considered as a preview of the work and the success obtained shall serve only to prove that utilization of the sorgo plant is not impossible and reopen the field of experimentation with this plant.

Notes on the Manufacture of Cane Sugar Syrups, Invert Sugar Syrup, Candy and Similar Products

(Prepared by the Sugar Technology Department of the Experiment Station)

The demand for candy, table and cooking syrups and molasses has increased tremendously in Hawaii along with the large increase in civilian and service population during the war. At the same time shipping space is at a premium. It should be possible to meet most, if not all, of this demand with local sugar and other products and thereby increase profits as well as reduce shipping space requirements between Hawaii and the mainland under war conditions. A resumé has been prepared touching upon the possibilities of manufacturing these products locally.

There does not appear to be any real reason or any great obstacle that should keep the Territory of Hawaii from being entirely self-dependent, in so far as products to satisfy its sweet tooth are concerned. Hawaii is an important source of sugar, both to ourselves and to the mainland of the United States. Sugar and the various products, which are or can be made from it, are a very important adjunct in the diet of all of our population. In one form or another it enters into a large share of the items of food which we consume. Many industries and many products depend upon sugar in one form or another; it is used in table and cooking syrups, molasses and invert sugar syrup, candy, bakery goods, beverages and many similar products.

Some products require sugar in its ordinary form as refined sugar, which is practically pure sucrose or as a pure sucrose syrup which is sometimes termed "neutral syrup." Other products require a mixture of sucrose and glucose in the form of a syrup. While glucose, which is a mixture of dextrose and levulose, occurs naturally to some extent in cane juices, it can readily be produced or its concentration increased in a sucrose-bearing syrup by one of several available inversion processes by which one molecule of sucrose becomes two molecules of glucose, or one each of dextrose and levulose. Still other products require the use of straight glucose or invert sugar syrup in which all sugars are in the form of dextrose and levulose. All of these ingredients to meet the requirements can be produced from local sugar and in conjunction with local sugar factories.

During the past years and even at the present time many tons of candy, table and cooking syrups and molasses, invert syrup or glucose and also yeast products from molasses have been manufactured on the mainland from sugar which could well be the identical sugar shipped over there from Hawaii and then these products shipped back into the Territory. This takes up valuable shipping space especially on the return voyage when this shipping space can, under present conditions particularly, be used for other essential needs.

Table syrups, cooking syrups and molasses can be made from raw cane juice evaporator syrup, raw, or washed raw sugars. Invert syrup or glucose can be made from raw, washed raw, or refined sugar. Candy can be made directly from the

same materials. To prepare the above items it requires but an inverting agent, such as a suitable acid or invertase to convert the sucrose or a portion of it in our products to glucose. Invertase can be prepared locally from ordinary yeast and it has already been demonstrated that yeast of a very high quality can be produced from our final molasses. This locally produced yeast could take care not only of the invertase needs, but also of the needs of the bakery industry in the Islands. A sufficient quantity could also be produced to supply materially the protein needs in food for humans and for livestock. As an acid-inverting agent large quantities of citric acid are already manufactured and are available from the pineapple industry, or if necessary it could be made from final molasses.

Thus we find that all of the necessary ingredients—crusher juice, evaporator syrup, raw, washed raw, or refined sugar, and final molasses—are available in large quantities to start production of all of the necessary saccharine products required by the confectionery, bakery, and beverage trades of the Islands. It appears that the production of these items for local needs is a logical step for the sugar industry. In most cases the additional equipment required would not be great, since practically all of the necessary equipment in one form or another is on hand at the factories.

Considerable work along this line has been carried on by the Sugar Technology Department on a laboratory scale and a fairly extensive search of the literature has been made. Instructions and formulas for various classes of candies and syrups from raw sugar syrup, raw sugar, washed raw sugar, and refined sugar have been prepared and were issued in the Department's Activities Report No. 4 of August 10, 1942. The candies include fudges, hard candies and brittles. It is assumed that most of the commercial quantities and qualities of candies will be taken care of by the new factory now being considered by the Hawaiian Pineapple Company.

The list of syrups includes table and cooking syrups and molasses, glucose or invert sugar syrup and "Hawaiian Sugar Honey." Considerable quantities of table syrup are now being produced by Honolulu Plantation Company, and several other plantations including Ewa, Oahu, and Hawaiian Commercial have for some time been making syrups for their employees or for sale through their plantation stores. These procedures, formulas, and additional data are presented herewith for general information.

The flavor and quality of these products will depend upon the care used and the method employed in their production and upon the initial product from which they are made. The desirability of each will depend upon individual tastes and the nature of the product for which it is to be used. Cooking, table, and invert syrups made from crusher juice or evaporator syrup will have a darker color and a stronger taste than those made from raw sugar and will also contain more of the mineral content and organic nonsugars. Raw sugar will in turn produce syrup with darker color and a stronger taste than washed raw or refined sugar. The color and flavor may be varied almost at will by the proper selection or blending of the starting materials. A light-colored, high-quality glucose or invert syrup would presumably be started from washed raw or from refined sugar or refinery syrup.

Discussion of various points in connection with these products and their manufacture will be undertaken in the following pages.

Syrups From Hawaiian Sugars

Syrup of excellent quality for table or cooking purposes can be produced locally provided containers can be secured for its storage and distribution. This syrup can be produced from cane sugar products by the use of either invertase or citric acid. Invertase or invertase yeast can be prepared locally. Citric acid is already produced in quantity by the local pineapple industry.

In general the flavor of table syrup made from washed raw sugar is more pleasing to the majority than that made from straight raw sugar, although if the syrup made from raw sugar is clarified somewhat by heating and settling or by filtration, the flavor is very similar to that made from washed sugar. There is a fairly large demand and sale for syrup made from crusher juice or evaporator syrup throughout the Territory.

Methods of making these plantation syrups differ and so do the flavor and color of the products. The flavor and color depend upon a number of factors in addition to the character of the materials from which the syrup is started and the method of making the syrup. If raw juice or crushed juice is used as the starting point, the juice is clarified by one of several methods, each of which may have some effect upon the final color and flavor.

At this point it may be of interest to cite some comments by H. S. Paine from the U. S. Department of Agriculture, Bulletin 1370, 1925, pp. 69–72:

The quality of flavor most desired in cane sirup is smoothness, with enough of the typical cane-juice flavor to give to it the unmistakable taste of the cane. Although the production of much ''caramel'' flavor during evaporation of juice to sirup is to be avoided, a little may improve the general character of cane sirup by making sweetness more noticeable and masking less desirable flavors. Cane sirup possesses more flavor than may at first be apparent. While this can be measured only by tasting, a method based upon the detection of flavor at various dilutions with water shows that in the average sirup the flavor is from 25 to 60 per cent more persistent than sweetness. The formation of small quantities of caramel and partial neutralization of the acidity of the sirup tend to equalize the intensity of sweetness and flavor.

Cane sirup owes its food value essentially to its sugar content.

The salts (ash constituents) and organic nonsugars in cane sirup have some incidental food value. Recent studies in nutrition and dietetics indicate that sugar-cane sirup and molasses contain a noteworthy quantity of vitamins. As the dietary value of the nonsugar substances is somewhat indefinite, however, the food value is usually calculated in terms of energy units on the basis of total sugars. Taking as a basis of calculation a fuel value of 3.749 large calories per gram for invert sugar and 3.955 large calories per gram for cane sugar, the energy value of one pound of cane sirup of the average composition . . . would be 1,188.6 large calories. One gallon of such sirup of [about 72° Brix] . . . weighing 11.35 pounds per gallon, has a food value of 13,491 large calories.

Sugar cane syrup if evaporated to a moderately high density will crystallize unless a portion of the sucrose has been inverted, while on the other hand a thin syrup is very likely to ferment in warm weather. Invert sugar does not readily crystallize. As the proportion of invert sugar to sucrose is increased, the likelihood of the syrup crystallizing is lessened. The problem is to make a syrup that will not crystallize when evaporated to a water content of about 20 per cent. Satisfactory syrups have been made in our laboratory having a density range of 72-78° Brix. Transformation of sucrose into invert sugar on a commercial scale can be brought about in several ways, but only those of commercial importance will be discussed. The two important procedures, inversion by heating with an acid and by

the action of an enzyme obtained from yeast called "invertase," will be discussed in some detail in the following sections.

Inversion Methods:

The methods employed for the conversion of the sucrose to glucose can be modified in their application and the degree of inversion can be controlled to meet the particular need. A system of control and adherence to certain details is essential to the success of the method employed and to obtain a high quality in the desired product. As stated above, two general methods are employed for inversion on a commercial scale; (1) heating with acids, and (2) the use of invertase. They will be discussed in their general aspects, including laboratory experience and comments gleaned from the literature on the subject, and then their application given in greater detail in reference to their use for specified products.

(1) *Inversion by Heating With Acids:* This method has been employed extensively. Hydrochloric or sulfuric are the acids most commonly used for the purpose, although tartaric, phosphoric and citric acids have been employed. All but the last of these are difficult to obtain in sufficiently pure form in the Territory at the present time as they are usually imported. Citric acid is produced locally and can be obtained in sufficient quantity for most purposes. Citric acid is very effective for the purpose, but as with all acids precautions must be observed in its use. The solubility of ordinary iron and copper is quite high in the presence of citric acid at the reactions and temperatures which are necessary. However, the amount of iron dissolved during the boiling has no noticeable effect upon the taste of certain syrups, and iron containers could be used for the inversion under some conditions. The amount of copper dissolved would affect the syrup or other product considerably and precludes the use of this metal until excess acidity is neutralized.

Some of the commercial methods employed using acids are described in the literature and may be of interest.

F. A. Lopez Ferrer describes a method which he uses in making invert syrup direct from cane. His article has been abstracted in *Facts About Sugar*, March 1936, p. 105, as follows:

The new industry of invert sugar manufacture has been of much value to certain mills in Cuba, as it has led to the utilization of cane that could not be used for the manufacture of crystal sugar, and in some cases has prolonged the grinding season 60 to 90 days. So far, sixteen Cuban factories have been making this product, and in 1935 they produced 54,468,283 gallons of invert syrup. The author is of the opinion that with continuance of restriction on sugar output, the amount of syrup that can be marketed at a good price is about 60,000,000 gallons.

In the process of manufacture supervised by the author, the operations are the same as for the production of crystal sugar up to the point of obtaining the thick juice (meladura), with the sole exception that the juice is kept as acid as possible, for the sake of economy in the inversion. No more lime is used in the juice than is required to produce a pH of 5.8 or 6.0, which corresponds to an inversion of 3 or 4 per cent when the juice is warmed up to more than 100° C. in the heaters and evaporators. The thick juice at 54° to 60° Brix is subjected to inversion with sulphuric acid in the proportion of 0.85 to 1.0 gallon of acid per 1,000 gallons of juice, which means 1.75 to 2.0 gallons of acid per 1,000 gallons of final invert syrup. Mixture of acid and thick juice is effected in six tanks provided with steam coils and compressed air. The mixing and heating must be done gradually and thoroughly to avoid caramelization. The first operation is called "active inversion"; it lasts 50 to 60 minutes and results in a syrup of 30 to 45 purity.

In the next operation, called "inversion in repose," the mixture is maintained at rest at a temperature corresponding to the working temperature in a vacuum pan. The thick juice is then concentrated under vacuum to 85° or 87° Brix. Before being discharged from the pan, the syrup is treated with one gallon of milk of lime (12° to 15° Baumé) per hectoliter of pan capacity, so that the pH on discharging will be about 6.8.

The author considers that the ideal invert syrup should contain 25 to 30 per cent real sucrose, and from 48 to 53 per cent glucose, which will give approximately 78 per cent of total sugars. The three factors of acid, time and temperature must be adjusted so as to secure maximum factory economy.

Incrustation of heating surfaces is more troublesome than in regular sugar manufacture, and metal surfaces should be treated with acid-resistant paint wherever possible, in order to diminish corrosion.

Another article on the production of inverted molasses in Cuba by D. G. Aurioles has been abstracted in *Facts About Sugar,* October 1937, p. 402, as follows:

The manufacture of inverted molasses (or invert syrups) though simple, requires select equipment and careful supervision. The technique of the process varies slightly according to the type of syrup used but always in agreement with the following rules: The Brix of the syrup varies from 72° to 84°, polarization after inversion from 20° to 42°. As an inverting agent, pure hydrochloric acid at 22° Baumé is used where the finest product is desired; the time of inversion varies from 20 to 40 minutes in the case of high purity syrups to 2 hours with inferior sugars. The final pH of the inverted syrup varies from 4 to 4.5, except when some customers wish a pH of 5.5 to 6.6, in which case the adjustment is made with milk of lime. In some mills phosphoric has been substituted for hydrochloric acid. Iron should not be used in the equipment, or the color of the product will be impaired.

In some cases, as in the preparation of a lower grade product, concentrated sulphuric acid is used for the inversion at the rate of 0.30 lbs. per 100 lbs. of syrup. The mixture is heated up to 200° F. and kept at this temperature until a rapid analysis shows a polarization of 0.10°; then milk of lime is added sufficient to bring the pH to 6.0. The syrup is then pumped to "waiting tanks" of the vacuum pans to be concentrated to 80°-85° Brix.

Another method which constitutes a practically continuous inversion and concentration process is described by J. R. Osuna in an abstract in *Facts About Sugar,* December 1940, p. 40, as follows:

The system devised by the author aims at effecting operations with as much convenience as possible. The process requires a heater, three cylindrical tanks, one or more vacuum pans, and a cooler. For the last, use may be made of a heater in which the cold water is circulated through the steam coils. The three tanks are connected with each other by 8″ piping and are used in rotation for inversion and mixing of the syrup.

The syrups are continuously concentrated in the vacuum pans, which are fed from the bottom; the concentrated syrup is continuously withdrawn from the pan by a pipe, which opens a foot above the top plate of the calandria and which is prolonged downward to a piston pump on the main floor of the factory. When the first or 'A' tank is one-fourth full, running in of the inverting agent (acid or yeast) is begun and it flows along with the syrup until 'A' is full. The filling of tank 'B' is then begun; half the contents of 'A' are transferred to tank 'C,' and when 'B' is full its contents are divided between 'A' and 'C,' which are discharged in rotation after completing the scheduled inversion time cycle.

A similar procedure is described by M. A. Mascaro and is abstracted in *Facts About Sugar,* April 1940, pp. 31–32, as follows:

Heretofore the procedure of making high test molasses (concentrated and acid-inverted cane juice) has consisted of mixing and heating the syrup and acid in a tank provided with a stirring apparatus and transferring the mixture to another tank for completion of the inversion cycle. To counteract the slowing down of the inversion process as the mass cools, it has been necessary to reheat it in the same tank by means of steam coils.

Experience has shown that this stationary heating in large tanks is detrimental because there is a loss of total sugars by caramelization and decomposition. For this reason the author has introduced a system which he describes as follows:

The juice follows the usual course until it reaches the quadruple effect, but is defecated with a minimum of lime, or with no lime at all if circumstances admit. The concentrated syrup (40°–42° Brix) is received in a tank for mixing with dilute acid in the usual manner. Then the mixture is pumped through the first of three small heaters of high speed design supplied with exhaust steam, where it is brought to 195°–200° F., and is discharged into tanks arranged in series. The acidified syrup remains in the tanks until its temperature falls to about 175° F., at which temperature the rate of inversion becomes too slow. The juice is then pumped through the second heater, to be again raised to the temperature at which inversion proceeds rapidly, and is discharged into a second series of tanks (or old crystallizers) where it remains until the purity has fallen to the point desired. The third heater is kept as a spare and for use during cleaning periods.

Considerable loss is avoided by this process. Even mills that obtain 79 per cent of total sugars may now obtain 80 to 81 per cent and perhaps more.

The California & Hawaiian Sugar Refining Corporation at Crockett produces an invert syrup for the beverage trade which demands a high-grade product. We have been informed that they use No. 1 concentrated liquor, which is one of the highest quality liquors, and that hydrochloric acid is used for the inversion. The requisite amount of acid is mixed with a definite amount of water in a rubber-lined tank. Inversion takes place in a glass-lined tank. A given amount of the liquor is run into the latter tank and the mixture of acid and water is added to this while the liquor is being agitated. This mixture is then gradually heated until a temperature of about 85° C. is reached and this temperature is maintained until the desired degree of inversion is accomplished. The resulting invert syrup is then brought to approximately the neutral point with caustic soda. The inversion of about 2,000 gallons of syrup can be accomplished in about an hour and a half with about 5½ pounds of hydrochloric acid.

Inversion can be accomplished in a similar manner with citric acid. It appears to require a slightly longer time to accomplish the inversion with citric than with hydrochloric acid. The required time will vary somewhat, depending upon the purity of the make-up syrup, lower purities requiring a longer time. If a particularly light-colored invert syrup is not required, the inversion can be carried out in iron tanks using any desired syrup, such as evaporator syrup or syrup made up from raw or washed raw sugar. The density should be in the neighborhood of 65° Brix and the inversion temperature about 85° C. As soon as inversion is carried to the desired point, the syrup is neutralized to about pH 6.2 with sodium bicarbonate or sodium carbonate. After the addition of the necessary amount of soda to neutralize excess acidity, the final concentration may be carried out in copper utensils. If a very light-colored invert syrup is required, the nature of the make-up syrup can be varied as well as the inversion procedure. Details of the inversion method will be described in a later section in connection with the various products discussed. On the basis of laboratory tests a thousand pounds of sugar, representing approximately 140 gallons of syrup at 65° Brix, would require about 9½ pounds of citric acid and 10½ pounds of sodium bicarbonate to give slightly over 110 gallons of invert syrup at 82° Brix.

(2) *Inversion with Invertase:* The use of invertase or a highly active invertase yeast is largely supplanting acids for the purpose of inverting sucrose to glu-

cose in the manufacture of table and invert syrups. It is generally conceded that invertase produces a better quality syrup with a better taste than the acid method and there is less corrosion of equipment. It is possible to produce in the laboratory a satisfactory invertase from yeast and there are several methods available for the production of high invertase-content yeast which can be produced in connection with any yeast plant. Some of the references, for the production of highly active invertase yeast, invertase and the use of invertase for syrup production, may be of interest at this point.

The use of invertase in connection with high-test invert molasses is commented upon by F. Guerrero, Proc. 13th Ann. Conf. Asoc. Tecnicos Azucareros, Cuba, 1939:

> The invert molasses industry has entered a new phase with the introduction of a yeast with powerful inverting properties, which enable the manufacturer to dispense with the use of acids that destroy sugar values and corrode the equipment.

A process used in Cuba is described by J. C. Gonzalez Maiz and his article has been abstracted in *Facts About Sugar,* March 1941, p. 30, a part of which follows:

> The process consists of delivering the sterilized syrup at the proper Brix and temperature to a vessel provided with arrangements for stirring and heating with steam. The required amount of yeast is added, and inversion is allowed to proceed until a polarization test shows zero, or better, minus 8 to minus 9 polarity. Then the syrup is again sterilized at 80° to 90° C. and concentrated in a vacuum pan to 85° Brix. The distillers, who are the principal consumers of inverted molasses, require a product that has been approximately 70 per cent inverted.
>
> With a Brix of 58°, a temperature of 58° C., and an inversion cycle of 12 hours the amount of invertase yeast consumed is 3.93 per cent on sucrose in syrup.

A process by which the yeast used for inverting syrup can be prepared at a factory is reported by A. P. Fowler and abstracted in *Sugar,* June 1941, pp. 37–38, as follows:

> It is generally conceded that inversion of sugar cane syrups by the use of yeast invertase is better than by the use of sulphuric or other acid. In the original Guerrero process, yeast for the purpose is propagated from pure cultures at the factory. An alternative is to purchase the yeast from manufacturers who specialize in its production on a large scale. The author offers a factory process as a middle course between the pure culture technique and purchase from outside sources. In this process 1500 gallons of a 6.5° Brix solution of well sterilized defecated juice is adjusted to a pH of 4.5 by means of sulphuric acid; some tri-calcium phosphate and nutritive salts are added and the solution is cooled to 86° F.
>
> A barrel (50 liters) of this solution is taken, treated with 70 cc. of concentrated sulphuric acid and 5 grams of sodium hydrosulfate, and the pH is brought to between 2 and 3 by addition of more sulphuric acid. Fifty pounds of stock or commercial yeast are broken up and well mixed in this solution, which is allowed to stand for at least one hour. Thereupon the main 1500 gallon wort is aerated at the rate of 300 cu. ft. of air per minute and the yeast mixture is added. The temperature is kept between 82° and 91° F., and the pH is adjusted to between 4.0 and 4.8 by addition of calcium carbonate when necessary. At the end of 8 hours the Brix of the wort should drop from 6.5 to 0.7, provided that air supply, pH and temperature have been properly maintained, and if there is no infection. At the end, the percentage of invertase content of the yeast should have increased five or six times over that in the original yeast. The milk of yeast thus obtained is added at the rate of 0.07 per cent of yeast paste (60% moisture) on sucrose to the syrup from the evaporators, which should have a Brix of not more than 60 and a temperature not exceeding 140° F. After that, the syrup is well mixed with the invertase yeast and kept at the proper temperature in the inversion tanks for the length of time permitted by the factory economics. In general, the apparent purity will drop from 80 to 10 in 12 to 15 hours and the inversion will amount to 60 or 70

per cent. The inverting ability of the build-up yeast is somewhat less than that of commercial invertase yeast prepared by specialists, but there is a substantial saving in cost.

Standards Brands, Inc., holds patents for the production of special yeasts of high inverting power.

Invertase of quite satisfactory quality has been extracted in our laboratory and methods for its use will be described in a subsequent section of this paper. This invertase has recently been used in making high-quality table syrup at one of our sugar factories.

Manufacture of Syrups by Acid Inversion Process

Formulas follow for making Table Syrup, Invert Syrup or Glucose, Hawaiian Sugar Honey, and Table and Cooking Molasses by the acid inversion method. Citric acid is used as the inverting agent in these formulas. The basic formulas for each are quite similar. The dissolved sugar material is heated to the boiling point, the source of heat removed and sufficient citric acid is added for the required amount of inversion which takes place during the period the material remains at high temperature. Sodium bicarbonate (baking soda) is then added to partially neutralize the acidity and the mixture boiled until the desired concentration is reached as evidenced by the specified final boiling temperature.

Each formula calls for an amount of sodium bicarbonate slightly in excess of the citric acid used, as we have found that it will be sufficient to remove the strong acid taste from the syrup but not enough to impair the taste from the use of too much soda. The pH before concentrating should be very close to 6.0.

Up to the point of partial neutralization with soda, almost any type of container except copper can be used. After adding the soda the acidity is so reduced that the boiling to final density can be made in copper containers if desired. It is preferable to concentrate the syrup under vacuum to avoid local overheating and possible impairment of the color and flavor due to too much caramelization. A formula for a table syrup which has a lower density than the other products with an end point of 108° C. (227° F.) will give a very palatable syrup if concentration is carried out either in the open or under vacuum.

All syrups if made in large batches should be run directly into the containers so that they will cool rather quickly. If finished syrup is to remain in a large kettle or tank for some length of time, provision should be made for its rapid cooling to protect the color and flavor, and when finally run into the containers the syrup should be reheated to about 175° F. to guard against mold growths.

Table Syrup:

The following proportions are those used for small-scale laboratory or home use, but they may be increased in the same proportion for large-scale use:

10 pounds raw sugar	10 pounds washed raw sugar
2 quarts water	2 quarts water
1½ ounces citric acid	¾ ounce citric acid
1¾ ounces sodium bicarbonate	⅞ ounce sodium bicarbonate

Dissolve the sugar in the water and heat to boiling. Remove from heat and stir in the citric acid which has been dissolved in a small amount of water. Keep at 85° C. or above for one half to three quarters of an hour or until an apparent purity

of about 45 is obtained on a sample diluted to 15–20° Brix. Stir in the sodium bicarbonate slowly and then boil with constant stirring to 108° C. (227° F.). If a little heavier syrup is desired, increase the final temperature one or two degrees. As a guide to operations on a large scale, the sugar should be dissolved in water and diluted to a density of about 65° Brix. The citric acid should produce a reaction of about pH 3.0–3.2 for the inversion. After the inversion is completed the soda should give a reaction of about pH 6.0. If vacuum concentration is used the end point is about 8° above the boiling point of water at any given vacuum.

Hawaiian Sugar Honey:

A very palatable and light-colored product that closely resembles commercial honey can be made from washed raw sugar and citric acid.

> 10 pounds washed raw sugar
> 2 quarts water
> ¾ ounce citric acid
> ⅞ ounce sodium bicarbonate

Dissolve the sugar in the water and heat to boiling. Remove from the heat and add the citric acid. Maintain the temperature at 85° C. (185° F.) or above for about 2½ hours or until the pol reading is negative in a sample diluted to 15–20° Brix. Then carefully add the sodium bicarbonate and evaporate under vacuum, to avoid caramelization and to protect the light color, until a boiling point elevation of 16° C. (29° F.) is reached. Break vacuum and run into containers. The Brix by refractometer is about 84°.

Table and Cooking Molasses:

This is a more highly flavored product than table syrup and is made from material representing the whole juice of the cane, in order to have it contain more of the flavor, color, mineral, organic and vitamin constituents than is obtainable when starting with raw, washed raw or refined sugar.

A convenient starting point for this product is after the juice has been clarified and is leaving the factory evaporators. This syrup is then partially inverted with citric acid to prevent crystallization at high density and then partially neutralized with sodium bicarbonate and finally evaporated to the desired density. The method of treatment would be as follows:

Heat the syrup to about 100° C. While stirring add one ounce of citric acid per gallon of syrup. Keep the temperature at about 85° C. or slightly above until a sample diluted to 15–20° Brix shows an apparent purity of 45–50. Stop the heat and while still stirring add 1¼ ounces of sodium bicarbonate per gallon of original syrup. It will probably be necessary to heat this syrup again to 100° C. and allow it to settle for from 2–6 hours to remove any sediment which may be formed. Most of the suspended material will settle out and the clear supernatent liquor can be decanted. The settlings can be filtered or returned to process via the liming tank and settlers. The clear syrup should be evaporated to the desired density, preferably under vacuum, with a boiling point elevation of 14° C. (25° F.). The density of the material made at the Station is about 84.3 refractometer Brix.

If the flavor of the product resulting from evaporator syrup is too strong, the batch may be made up before inversion using one-half evaporator syrup and one-

half raw sugar dissolved to the syrup density, or any other portions of raw or washed raw sugar may be used to obtain the desired flavor.

Glucose Syrup or Invert Syrup:

Glucose syrup is made by the inversion of all or nearly all of the sucrose. A longer time for more complete inversion of the sucrose to glucose is allowed than when making table syrups. Fairly complete inversion is accomplished by the following procedure:

> 10 pounds raw sugar
> 2 quarts water
> 1½ ounces citric acid
> 1¾ ounces sodium bicarbonate

Dissolve the sugar in the water and heat to boiling and add the citric acid. Keep at 85° C. (185° F.) or above for 5 hours or until a negative pol reading is obtained when a sample is diluted to 15–20° Brix. The closer the temperature is kept to 100° C. (212° F.) the more rapid the inversion will be. When a negative reading is obtained stir in the sodium bicarbonate slowly as the mixture will foam considerably. It is best to do the final boiling under vacuum with a final temperature elevation of 16° C. or 29° F., as this material will caramelize very easily at the higher densities.

CANDY FORMULAS

Plain Fudge:

(LARGE BATCH)	(SMALL BATCH)
10 pounds raw or washed sugar	1½ pounds raw or washed sugar
2 quarts water	¾ pint water (1½ cups)
¼ ounce citric acid	½ gram citric acid (⅛ teaspoon or a little less than the amount you can heap on a dime)

Dissolve the citric acid in the water and add the sugar. Heat and boil until a thermometer indicates a final temperature of 115.5° C., or 240° F. Cool to 65° C., or 149° F., then stir or beat until change to dull appearance occurs as it starts "sugaring." Then quickly pour on a greased slab or pan. Cool and cut into squares. Flavoring may be added if desired after the final temperature has been reached. However, the original flavor of raw sugar changes during the boiling and the candy has a flavor resembling that of maple sugar.

Chocolate Fudge:

(LARGE BATCH)	(SMALL BATCH)
10 pounds raw sugar	1½ pounds raw sugar
2 quarts water	¾ pint water (1½ cups)
⅛ ounce citric acid	½ gram citric acid (⅛ level teaspoon)
1⅓ pounds chocolate	¼ pound chocolate

Dissolve the citric acid in the water and add the sugar and chocolate. Heat slowly to boiling and boil to 114° C., or 237° F. Cool to 63° C., or 146° F., then stir until change to dull appearance occurs and pour on greased marble slab or pan. Cool and cut into squares. If flavoring is to be added, put in when boiling is completed. Nuts may be added just before pouring on slab. The amount of chocolate can be varied to suit individual taste.

Hard Candy:

(LARGE BATCH)	(SMALL BATCH)
10 pounds raw sugar	1½ pounds raw sugar
1 gallon water	1 pint water (2 cups)
¼ ounce citric acid	1 gram citric acid (¼ level teaspoon)
1 level teaspoon salt	⅛ teaspoon salt

Dissolve the citric acid in the water and add the sugar and salt. Heat slowly to boiling and boil to 147° C. (297° F.). Nuts may now be added and the mixture poured on a greased marble slab or pan. Spread out thin with a spoon or spatula. Cool, break into pieces and store in airtight jars or moisture-proof containers.

Peanut Brittle:

(LARGE BATCH)	(SMALL BATCH)
10 pounds raw sugar	1½ pounds raw sugar
1 gallon water	1 pint water (2 cups)
¼ ounce citric acid	1 gram citric acid (¼ level teaspoon)
1 level teaspoon salt	¼ teaspoon salt
1½ ounces sodium bicarbonate	1 level teaspoon sodium bicarbonate
6 pounds shelled, roasted or unroasted peanuts	1 pound shelled, roasted or unroasted peanuts

Dissolve the citric acid in the water and add the sugar and salt. Heat to boiling and boil to 147° C. (297° F.), stirring frequently to prevent caramelization. Remove from heat source and add the sodium bicarbonate (baking soda) and stir quickly and thoroughly. Now add the peanuts, mix thoroughly and pour on a greased marble slab or pan. Spread out thin with a spoon or spatula. When cool, break into pieces and place in airtight or moisture-proof containers.

Manufacture of Syrups by the Invertase Process

If mill juice or crusher juice is used, it must be clarified by any of several methods and then evaporated to a density suitable for the inversion or to about 37.5 to 40° Brix. Evaporator syrup can be used by either taking it off at a lighter than ordinary density or else it should be diluted with water or clarified juice to the desired density. Raw sugar, washed raw sugar or the washings from washed raw sugar may be used. If raw or washed raw sugar is used, it is dissolved and diluted to the proper density.

Several hours must be allowed for inversion. After sufficient inversion has taken place, the inverting action is stopped by heat or neutralization and the syrup is then evaporated to a heavy-bodied finished syrup. Under certain circumstances it may be necessary to filter the syrup before concentrating or to heat to 100° C. and allow to settle. The density of the solution, temperature, reaction and the amount and activity of the invertase determine the time required for the inversion and are the four important factors in the control of inversion in syrup making. The final sucrose purity desired is usually between 45 and 50.

Density for Inversion: Invertase acts rapidly upon sucrose in dilute solutions, but in concentrated liquors the rate of its reaction is greatly reduced. It becomes materially reduced above 40° Brix and for the most satisfactory work the density should be about 36° Brix when tested at 58–60° C.

Temperature for Inversion: The optimum temperature for inversion will vary slightly with different batches of invertase, but will usually be in the neighborhood of 55–60° C. (135° F.). Invertase is rapidly destroyed as the temperature is in-

creased above 60°, therefore the temperature should be held as near that point as possible but not above it. For practical operation the inverting tanks should be sufficiently insulated so that the temperature drop is not more than 10 degrees for the first 12 hours. The invertase should be added after the syrup has been heated to 60°. The syrup should be thoroughly mixed before and after adding the invertase. If it should be necessary to reheat during the course of inversion, it should never be with open steam coils and the syrup must be thoroughly stirred to prevent any local overheating. However, if invertase is added to a large volume of syrup in a well-insulated tank, the cooling will ordinarily be so slow that, for all practical purposes, the temperature will remain high enough during the time necessary for the required amount of inverting action to take place.

Reaction (pH): Invertase exerts its greatest activity in a slightly acid solution. The optimum reaction is usually at about pH 4.8 to 5.2. If the syrup is made by heating and skimming raw or crusher juice without the addition of lime, the natural acidity will be just about right for optimum inversion and no adjustment will be necessary. If clarified juice, syrup, or sugar is used the reaction must be adjusted to between pH 4.8 and 5.2. Any pure acid can be used for this purpose. Citric acid, being produced locally, can be used since the required reaction is not low enough and the temperature not high enough to cause damage to the equipment.

Time for Inversion and Amount of Invertase: The time for inversion to take place and the amount of invertase required are dependent one upon the other, together with the factors already mentioned. Other factors remaining constant, the inversion will be faster with larger quantities of invertase. Conversely, the amount of invertase can be reduced if sufficient time is available for a slower inversion. Capacities are usually calculated to allow from 12 to 16 hours for inversion.

If the activity of any particular batch of invertase is not known it should be determined under controlled temperature conditions. The following example will illustrate the activity of a batch of invertase prepared at the Station:

Washed raw sugar syrup having a purity of 99.0 and a density of 38.6° refractometer solids was used for this test. The activity was determined at varying reactions and at different temperatures, *i.e.*, reactions of pH 4.6, 5.0 and 5.4 and temperatures of 52, 58 and 64 C. Invertase was added at the rate of 1 ml. per pound of sugar in solution. The average drop in purity per hour at 58° C. was 5.6 at pH 4.6, 5.5 at pH 5.0, and 5.0 at pH 5.4. This indicated the optimum reaction to be between pH 4.6 and 5.0. The average purity drop per hour for different temperatures was 4.4 at 52°, 5.3 at 58° and 5.8 at 64°. While the indicated rate was higher at 64, the temperature was actually too high, for the bihourly figures showed that the invertase was being destroyed. The drop was 6.75 for the first two-hour period, 5.8 for the second two-hour period and 5.0 for the third two-hour period. The figures actually showed that the optimum activity was obtained at a reaction of pH 5.0 and a temperature of 60 C. Under these conditions the average purity drop per hour was 6.0+.

From the above data the requirements for a 500-gallon tank of syrup can be estimated as follows: 500 gallons of syrup made from washed raw sugar at 38.5 Brix and 98.5 purity would contain approximately 1840 pounds of sucrose or sugar in solution. If it is required that this be inverted from 98.5 purity to 48.5 purity the total purity drop is 50 points. Under the conditions of the above test, 1 ml. of invertase per pound of sugar in solution at pH 5.0 and 60° C. would produce a

purity drop averaging about 6.0 points per hour. Therefore, if invertase were added in the same proportions and the temperature maintained at 60° C., 8½ hours would be required. If 17 hours could be allowed for the inversion, the invertase requirement could be reduced to one half the quantity. The usual practice in syrup plants is to start the inversion about 4:00 o'clock in the afternoon and allow it to proceed overnight for a total of about 16 hours. Allowing for a temperature drop of from 8 to 10 degrees, the amount of invertase could be reduced by about one quarter or from 1840 ml. to about 1400 ml. and the amount of inversion should still be sufficient. Inversion will be still more rapid if the density of the syrup is reduced to about 35° Brix, but at the same time this means more water to be evaporated.

Directions for Inversion with Invertase: Evaporate clarified cane juice rapidly to about 35° Brix at the evaporator temperature or dilute finished syrup, washed or unwashed raw sugar, to the same density and run it into the inversion tank. Adjust the reaction to pH 5.0 and the temperature to 60° C. (140° F.). Add the required amount of invertase to produce the desired degree of inversion in the allotted time. Then mix thoroughly with air or by mechanical means. If the insulation is sufficient, the solution may be allowed to stand for the necessary time without further attention. If it is necessary to reheat during inversion it should not be done with an open steam coil and great care should be exercised to prevent any local overheating around the heating units.

These instructions should be followed as closely as possible, but slight variations will not greatly affect the results. For instance a variation of two or three degrees in Brix is permissible and a similar difference in temperature is allowable. Also the reaction range may vary from 4.6 to 5.2 pH without affecting the results to a material extent.

The course of the inversion should normally be followed by "purity" or "pol" determinations about every three hours. This is not necessary except to determine the degree of inversion near the end of the cycle. However, such a routine if it can be followed will indicate whether inversion is proceeding as it should and if not, allow the operator to correct the difficulty before too much time has been lost. If the above conditions are maintained it should be possible to reproduce the results from day to day with the same batch of invertase. It may be necessary to change the temperature or the reaction slightly for different batches of invertase.

Inversion is stopped either by heating to 75°–80° C. or by neutralization. Lime is the most economical neutralizing agent. Neutralization is optional; the unneutralized syrup has a slightly sharper taste which some people prefer. Neutralization tends to equalize the intensity of sweetness and flavor and produce a somewhat smoother product.

Filtration: It will usually be found necessary to filter the syrup after inversion or to heat it and allow it to settle to secure a clear syrup. It may also be necessary to filter after final evaporation. The addition of a very small amount of phosphate in the form of Ammo-phos followed by lime to a reaction of about pH 7.6 effects a marked clarification and does not give any difficulty in the filtration after inversion has been completed and the syrup heated to 75–80° C. Filtration is preferred to settling if the equipment is available. A second filtration is sometimes practiced after evaporation to final density. If filter aid is available it will materially assist filtration.

A secondary precipitation has occurred in some instances after the final syrup has stood for some time. The cause of this has not been definitely determined, but we have been informed from one source that this has been overcome by heating the heavy syrup to not over 75° C.

Evaporation to Final Density: Evaporation may be accomplished under vacuum or by "open-kettle" boiling at atmospheric pressure. The choice at present would depend largely upon the equipment available. Evaporation under vacuum is more readily controlled and is conducive to a more uniform product. The two methods produce syrup with some differences in color and flavor; the open-kettle method is usually accompanied by some caramelization, is slightly darker in color and has a slightly sharper flavor.

Density: The standard commercial density is about 72° Brix. Some prefer a slightly lower or a slightly higher density. Samples of syrup produced locally range from 69 to 75° Brix. Syrup does not readily ferment in warm weather at 78–80° Brix, but proper canning or bottling is depended upon to prevent fermentation at densities around 70–72° Brix. Practically no fermentation has been observed in samples of locally produced table syrup.

Purity: Reduction in apparent purity to between 50–55 is sufficient to prevent crystallization at a density of approximately 72° Brix, but if it is desired to carry the density at 78–80° Brix the purity should be reduced to about 45.

Cooling Syrup: Syrup should not be placed in storage tanks while hot, but should be cooled somewhat during its passage from the evaporators to the storage tanks. The temperature should be reduced to at least 180° F., or about 82° C., rather rapidly after evaporation if it has been concentrated by the open-kettle method to prevent any change in color or flavor.

Canning Syrup: The canning of table syrup is, fortunately, not a difficult operation, but the subject is worthy of more discussion than can be devoted to it here. It is fully covered in U.S.D.A. Bulletin 1370, 1925, p. 58, in a section by W. L. Owen, from which the following is taken:

Cane sirup may be preserved in cans [or bottles] with little chance of failure, if certain simple rules are observed. There are no great difficulties to be overcome, and there is no reason why, even with the simplest equipment, cane sirup can not be canned with minimum loss from spoilage. The three general conditions necessary are (1) to fill the can with sirup at the proper temperature [170° to 180° F.], (2) to obtain airtight closure of the can, and (3) to avoid long retention of heat by the sirup both before and after canning.

Glucose or Invert Syrup Made with Invertase: Complete inversion of all of the sucrose, to form glucose or invert sugar in the production of glucose or invert syrup, can be accomplished by the use of invertase in a manner similar to that just described. In order to produce a high quality of invert syrup it would be necessary to start from washed raw sugar or even from refined sugar. Other steps in the process would not be greatly changed, except that the inversion would be carried to a minus polariscope reading of about 17°.

* * * * *

U.S.D.A. Bulletin No. 1370, 1925, contains a compilation of valuable information on the subject "Sugar-Cane Sirup Manufacture" and can be procured from The Superintendent of Documents, Government Printing Office, Washington, D. C., for 10 cents per copy.

Preparation of Invertase for Syrup Making

Invertase with reasonable inverting power (activity) may be prepared from ordinary compressed yeast as follows:

Method in Brief: Break up five pounds of compressed yeast into a wide-mouthed container. Add 30 ml. of chloroform, cover with a cloth and allow to stand for 36 to 48 hours at room temperature. This mass is then filtered and the filtrate stored in a refrigerator. If a refrigerator is not available, toluene may be poured on top to form a layer of about ⅛ inch.

Details of Method: The following details have been learned from our experience in the Station laboratory and may be of some value.

Invertase with quite satisfactory activity has been prepared from compressed yeast produced in the Station's pilot plant.

The chloroform should be quite well distributed over the surface of the yeast. It may even be mixed into the yeast with a stirring rod or stick although this is not necessary.

After a varying length of time the mass begins to liquify and become fluid. This time varies from an hour and a half up to five or six hours and seems to depend upon the age of the yeast and the temperature. At this stage the entire mass should be gently stirred and mixed to break up lumps and form a uniform mixture. Also at this stage the mass may foam or froth considerably and if not watched or if in too small a container a large portion may be lost. Foaming over can be prevented by gently stirring down the foam occasionally. Too vigorous stirring of the entire mass may cause more foaming.

When the first vigorous frothing has ceased, add about 200 ml. of water and gently mix into the mass and allow it to stand. There is usually then no further danger of foaming over.

Use a container of ample size to allow for from three to five times the volume occupied by the crumbled yeast. A wide container with wide mouth is preferable. An earthenware crock is suitable. At the Station a four-gallon enameled ware cooking pot with a ratio of 1:1 or 1:3 diameter to height is used for five-pound lots of yeast, a six-gallon container for ten-pound lots and a ten-gallon container for 20-pound lots.

The procedure at the Station is to mix the yeast and chloroform about noon, then the dangerous stage of frothing is over by closing time and the mass is allowed to stand until the second morning following when filtration is started.

A number of methods have been tried in attempting to effect a rapid separation of the liquid from the mass of yeast cells, for this filtration is very slow. Attempts with suction, pressure or centrifuge have not been satisfactory. The following procedure is now used: Six to a dozen flasks with 6-inch funnels are fitted with 8- to 12-inch fluted filter papers. A cheap grade of coarse filter paper is permissible. These are filled and allowed to drain most of the day. The material remaining on the filter papers is poured back into the original container, the funnels fitted with new papers are again filled and these are allowed to drain overnight. The same procedure is followed the next morning if necessary, or, until the entire mass is filtered. The heavy mass remaining on and clinging to the filter paper with the paper itself is accumulated in a separate container. To this is added a volume of water equal to the volume of filtrate which was extracted and about 10 ml. of chloroform. The mass of yeast and filter paper is broken up and stirred into a homo-

geneous mass, allowed to stand overnight and filtered in the same manner as the original material. This filtrate is mixed with the main filtrate and placed in the refrigerator.

The filtrate, of course, contains the invertase. It may be further purified and concentrated, but this is a long tedious process and appears unnecessary for present purposes. The activity (inverting power) of this crude invertase is not as high as the commercial product purchased on the market, but it serves as a very good substitute under present conditions. The optimum temperature and reaction may vary somewhat in different batches. Therefore, it seems preferable to prepare fairly large batches and then determine the optimum temperature in the laboratory and apply these findings in the plant.

An example of the activity of a batch of about 20 liters of crude invertase prepared in this laboratory follows:

Washed raw sugar syrup at 99° refractometer purity and 38.6° refractometer solids was used in the test. Invertase was added in the proportion of 1 ml. per pound of sugar.

Purity drop	Different reactions at a temperature of 58° C. —pH—			Different temperatures at a reaction of pH 5.0 —T° C—		
	4.6	5.0	5.4	52	58	64
2 hours	11.6	11.2	11.3	9.1	11.4	13.5
4 hours	22.4	22.4	21.6	17.6	22.4	25.2
6 hours	32.6	32.6	30.1	25.5	32.1	35.2
Average drop per hour....	5.5	5.5	5.0	4.3	5.4	5.8

These figures show little difference in the activity at reactions of pH 4.6 or 5.0, but do indicate a slight dropping off at pH 5.4. The figures show a decided increase in activity with increased temperature from 52° to 64° for the first two-hour period, but the increase is not sustained at 64° for the 4- and 6-hour periods, and indicate that 64° C. is somewhat too high for this invertase. The optimum conditions for this batch would then be a reaction of pH 5.0–5.2, (maintaining the higher reaction to conserve acid and reduce corrosion of equipment) with a temperature of 60° C. These conditions should then give a purity drop of about 6 points per hour. With these data the necessary amount of invertase to invert a given amount of sugar in a given time could be estimated, or conversely, the time interval required for a given amount of sugar and a given amount of invertase could be calculated.

Proper temperature control is the most important factor in obtaining the maximum activity in inversion with invertase. The invertase should be added to the syrup *after* the syrup has been brought to the desired temperature, and if it is necessary to apply heat during the course of the inversion it should not be from open steam coils and care should be taken to insure thorough mixing to prevent local overheating.

The subject of invertase preparation has not been given a great deal of study at the Station and, doubtless, the method and technique herein described can be considerably improved. However, invertase prepared as above has been used to produce a very satisfactory syrup and definite progress is being made toward filtration of the treated yeast in a filter press.

References on Invertase Preparation:

Handbook of Sugar Analysis—Browne, C. A., 1st Edition, pp. 669–670, 1912.

Sugar Analysis—Browne, C. A., & Zerban, F. W., 3rd Edition, pp. 428–437, 1941.

The Synthesis of Sucrose in the Sugar Cane Plant*—I

By CONSTANCE E. HARTT

In providing for its own nourishment and the growth of its own body, the noble sugar cane plant is wont to carry its sugar in transit and in storage in the superior form of sucrose. In recent years, however, the sugar canes cultivated in Hawaii do not seem to be maintaining as high a standard in this respect as formerly but are inclined to carry more and more of their sugar in the form of glucose and its counterpart fructose. As a result of this delinquency, the trend in the quality of our sugar cane juices is downward and we are unable to recover as much sucrose therefrom as we desire. Since we have proved that the formation of glucose precedes the formation of sucrose in the sugar cane plant, studies to determine the factors influencing the conversion of glucose to sucrose may point the way to means of improving the quality of our sugar cane juices.

The transformations of sugars in the leaves of plants have been studied by several investigators. Russian physiologists have been particularly active, and many of their results have recently been summarized (49)†. Other investigators active in this field include Virtanen and Nordlund (83), Nurmia (née Nordlund) (65), Leonard (51), and Hassid (36, 58). No attempt will be made to review their results in this report, but their works are cited for the benefit of anyone who might wish to learn the scope of the investigations being carried on elsewhere in this particular field of research.

Our studies of the synthesis of sucrose by excised blades of sugar cane have been carried on over a period of more than five years and two reports (33, 35) have already been published. Our investigations are not yet completed, but it seems advisable at this time to record our progress to date in permanent form. For the sake of convenience, this report is divided into four parts. The first deals with sugar transformations in separate organs, entire stalks, and entire plants; also with time, temperature, chlorophyll, aeration and mutilation. The second part deals with the effects of several inorganic and organic compounds. The third part deals with specific inhibitors. The fourth part deals with the interrelationships of the factors treated in the first three parts and an attempt is made to explain the sequence of events in the synthesis of sucrose.

The results lead to the conclusion that the formation of sucrose fits into the general scheme of carbohydrate metabolism already established by the studies of Cori (10–12), Hanes (29), and others. Because inhibiting the formation of fructose diphosphate inhibits the formation of sucrose, whereas inhibiting the breakdown of fructose diphosphate increases synthesis, we are led to conclude that fructose diphosphate is a stepping stone in the formation of sucrose by the sugar cane plant.

---

† Numbers in parentheses refer to literature citations at the end of the fourth part of this paper.

The Hawaiian sugar cane varieties 109 and 32–8560 were used in this investigation. Tests showed that these varieties are equally suitable for these studies. In the experiments using blades, the plants were grown in the field at the Experiment Station and had received optimum fertilization and irrigation. In the experiments using roots, the plants were grown in complete nutrient solution and with aeration in tanks of approximately 25 gallons capacity. Blades were cut from the plants in the morning, and were always taken from the same position on the plant. Generally eight blades were used in each series. Roots were shaved from stems, washed, dried in a centrifuge, and weighed—these procedures being conducted uniformly for each experiment.

Samples of blades and roots were ground at once and preserved for analysis, these samples being designated "initial controls." The other series were placed in their respective solutions, in a constant temperature room in absolute darkness. After the experimental period, generally 24 hours, they were washed, dried, ground, and sampled.

The methods of sampling and analysis were the same as those previously described (35).

All of the results of the moisture determinations are expressed as percentages on the wet-weight basis; all of the results of the sugar determinations are expressed as percentages on the dry-weight basis unless otherwise specified.

PART I

THE INTERCONVERSION OF GLUCOSE AND FRUCTOSE AND THE FORMATION OF SUCROSE IN DETACHED ORGANS OF THE SUGAR CANE PLANT

1. The formation of sucrose from glucose:

Many tests have been conducted in which blades detached from the plant have been placed with their cut ends in five per cent solutions of glucose. The blades absorb some of the glucose and increase in percentage of sucrose. The results of a typical test are recorded in Table 1 which shows that blades supplied with glucose gained considerably more sucrose than reducing sugars. This gain in sucrose is considered to be an actual synthesis of sucrose from the glucose supplied, rather

TABLE I
MOISTURE AND SUGAR PERCENTAGES OF BLADES SUPPLIED WITH 5% GLUCOSE FOR 24 HOURS

Series	Moisture	Reducing sugars	Sucrose	Total sugars
Initial control	68.07 ± 0.076	0.817 ± 0.027	2.905 ± 0.014	3.875 ± 0.012
5% glucose	63.61 ± 0.024	1.820 ± 0.029	6.452 ± 0.006	8.612 ± 0.036

than a rearrangement of the carbohydrates already within the blades, because similar blades supplied with an inorganic solution of the same osmotic concentration as the glucose showed no increase in sucrose (33).

The formation of sucrose from glucose can also take place in sheaths, as shown in Table II. The blades and sheaths mentioned in Tables I and II were taken from the same stalks at the same time, and the results may therefore be compared. A convenient method of comparison is afforded by the synthetic efficiency, which is the percentage of glucose absorbed that is converted into sucrose. The synthetic effi-

TABLE II

MOISTURE AND SUGAR PERCENTAGES OF SHEATHS SUPPLIED
WITH 5% GLUCOSE FOR 24 HOURS

Series	Moisture	Reducing sugars	Sucrose	Total sugars
Initial control	75.56 ± 0.195	3.715 ± 0.001	7.777 ± 0.019	11.900 ± 0.002
5% glucose	74.62 ± 0.257	3.986 ± 0.002	9.870 ± 0.067	14.380 ± 0.071

ciencies of these blades and sheaths, calculated from Tables I and II, are found in
Table III. Although the sheaths gained less total sugar and sucrose than the blades,
the process of synthesis operated fully as well, in fact better, in the sheaths than in
the blades.

TABLE III

THE SYNTHETIC EFFICIENCY OF BLADES AND SHEATHS,
CALCULATED FROM TABLES I AND II

Series	Gain in total sugar %	Gain in sucrose %	Synthetic efficiency
Blades	4.737	3.547	74.87
Sheaths	2.480	2.100	84.67

The formation of sucrose from glucose was also studied in the stem. The upper
portion of the stem was used, from which all leaves were removed. A wild variety
of sugar cane, *Sacharum robustum* (Molokai 1293) from New Guinea, was used
in these tests, because the cultivated or noble canes already contain so much sugar
that they cannot absorb any more. The results are presented in Table IV. The
synthetic efficiency of the stems was 45.31. It is evident that sucrose can be made

TABLE IV

MOISTURE AND SUGAR PERCENTAGES IN STEMS SUPPLIED
WITH 10% GLUCOSE FOR 48 HOURS

Series	Moisture	Reducing sugars	Sucrose	Total sugars
Initial control	82.16 ± 0.410	5.139 ± 0.256	5.23 ± 0.067	10.64 ± 0.181
10% glucose	83.78 ± 0.105	6.810 ± 0.032	6.68 ± 0.048	13.84 ± 0.029

from glucose in the stem of sugar cane, but that this process is not as efficient in
the stem as in the blade and sheath.

The results of studies with entire stalks are presented in Table V. Stalks of
variety *Saccharum robustum* (Molokai 1293), age 1½ years, were cut at the joint

TABLE V

MOISTURE AND SUGAR PERCENTAGES IN ENTIRE STALKS OF *SACCHARUM
ROBUSTUM*, SUPPLIED WITH 10% GLUCOSE FOR 48 HOURS

Series	Moisture	Reducing sugars	Sucrose	Total sugars
Blades:				
Initial control	66.57 ± 0.091	0.571 ± 0.018	1.175 ± 0.019	1.808 ± 0.039
10% glucose	65.40 ± 0.835	1.089 ± 0.050	3.096 ± 0.187	4.349 ± 0.247
Sheaths:				
Initial control	77.46 ± 0.162	1.925 ± 0.251	1.803 ± 0.091	3.824 ± 0.347
10% glucose	75.32 ± 0.386	2.779 ± 0.266	5.368 ± 0.021	8.430 ± 0.288
Stems:				
Initial control	82.16 ± 0.410	5.139 ± 0.256	5.23 ± 0.067	10.64 ± 0.181
10% glucose	76.73 ± 0.696	5.531 ± 0.408	12.58 ± 0.720	18.78 ± 0.353

at which the lowest living green leaf was attached, and were placed in 10 per cent solutions of glucose. There were three stalks per series, and each series was run in duplicate. The results in Table V are the averages of these duplicate series. After 48 hours the blades, sheaths, and stems were separated and sampled. The percentage of sucrose more than doubled in all three organs. The question arises, as to whether the increase in sucrose in the stem is due to synthesis *in situ* or to translocation from the leaves. This question may be answered by reference to Table IV, in which excised stems of the same plants as those in Table V were used. The results with excised stems show that sucrose can be synthesized from glucose in the stem, but the amount made in that way by no means accounted for all of the increase in sucrose in the stems of the entire stalks. Therefore both synthesis *in situ* and translocation from the leaf are involved in the storage of sucrose in the stem.

The absorption of glucose and formation of sucrose were next studied in entire plants. For this purpose we used plants of 2–4 months of age, which had been grown in complete nutrient solution. The plants were placed with their roots in five per cent solutions of glucose for 48 hours, with fresh glucose supplied after 24 hours. The results of a typical test with three plants per series are recorded in Table VI, which shows that the roots absorbed glucose and made sucrose. In the

TABLE VI

MOISTURE AND SUGAR PERCENTAGES IN ENTIRE PLANTS SUPPLIED WITH 5% GLUCOSE FOR 48 HOURS

Series	Moisture	Reducing sugars	Sucrose	Total sugars
Blades:				
Initial control	73.81 ± 0.100	2.227 ± 0.007	4.195 ± 0.000	6.643 ± 0.006
5% glucose	73.80 ± 0.100	3.792 ± 0.003	2.390 ± 0.001	6.308 ± 0.001
Sheaths:				
Initial control.........	82.37 ± 0.038	7.154 ± 0.006	8.867 ± 0.057	16.488 ± 0.065
5% glucose	79.81 ± 0.029	10.151 ± 0.013	5.762 ± 0.017	16.217 ± 0.031
Stems:				
Initial control	84.47 ± 0.081	9.863 ± 0.048	21.113 ± 0.009	32.087 ± 0.058
5% glucose	82.96 ± 0.038	9.795 ± 0.030	22.804 ± 0.056	33.799 ± 0.089
Roots:				
Initial control	88.99 ± 0.024	4.427 ± 0.120	6.439 ± 0.086	11.204 ± 0.211
5% glucose	88.50 ± 0.091	8.097 ± 0.016	10.639 ± 0.048	19.296 ± 0.035

blades and sheaths there were no gains in sucrose, and in the stems the gain in sucrose was much smaller than in the roots. The failure of the tops to gain in sucrose was typical of these experiments with entire plants, whereas when stalks were used, considerable increases in sucrose occurred in blades, sheaths, and stems, as shown in Table V. It would seem that translocation of sugar from roots to stems was hindered in some way. Since it is well known that roots ordinarily obtain their supply of sugar from the tops, these results suggest the existence of polarity in translocation of sugar from tops to roots.

The data recorded in Table VI may be taken to indicate that the synthesis of sucrose from glucose can take place in roots. To study this question further, an experiment was conducted in which both attached and detached roots were used. The results are presented in Table VII. Since the attached roots made considerably more sucrose than the detached roots, it was concluded that some substance essential for synthesis was supplied from the tops to the roots (34). Studies of the effects

of aeration, of vitamins, and of hormones were therefore undertaken, and are described elsewhere in this report.

TABLE VII

MOISTURE AND SUGAR PERCENTAGES IN ATTACHED AND DETACHED
ROOTS SUPPLIED WITH 5% GLUCOSE FOR 48 HOURS

Series	Moisture	Reducing sugars	Sucrose	Total sugars
Initial control	90.27 ± 0.219	0.275 ± 0.029	2.539 ± 0.053	2.948 ± 0.085
Attached roots	90.25 ± 0.138	3.291 ± 0.060	4.214 ± 0.088	7.728 ± 0.032
Detached roots	90.89 ± 0.052	7.113 ± 0.019	2.803 ± 0.037	10.064 ± 0.020

The experiments already described lead to the conclusion that the formation of sucrose from glucose can take place in blades, sheaths, stems, and roots of the sugar cane plant, when supplied with glucose. This process takes place in absolute darkness. To compare this process with the formation of sucrose in photosynthesis, detached blades were placed in water in sunlight for 11½ hours, and similar blades were supplied with 10 per cent glucose in the dark for the same length of time. For this test, blades were taken from different levels on the plant. Counting the leaf with the highest visible ligule as leaf number 1, our routine method is to use leaves 1 and 2 in these studies. For this particular test, we compared leaves 1 and 2 with older leaves, numbers 7 and 8. The results are recorded in Table VIII. Blades 7 and 8 increased in sucrose more than blades 1 and 2, whether by photosynthesis or by synthesis in the dark. The difference was about the same: by photosynthesis, 0.493; and by synthesis in the dark, 0.644. This is in agreement with the theory that the synthesis of sucrose takes place by the same mechanism whether the glucose is supplied artificially as in these tests or naturally by the process of photosynthesis. Further evidence that we are dealing with the natural process of sucrose formation is afforded by other experiments in this report.

TABLE VIII

MOISTURE AND SUGAR PERCENTAGES IN DETACHED BLADES IN WATER
IN SUNLIGHT, AND IN BLADES SUPPLIED WITH 10%
GLUCOSE IN THE DARK, FOR 11½ HOURS

Series	Moisture	Reducing sugars	Sucrose	Gain in sucrose	Total sugars
Initial controls:					
Blades 1 & 2.....	73.01 ± 0.057	1.621 ± 0.010	3.913 ± 0.036		5.740 ± 0.028
Blades 7 & 8.....	72.60 ± 0.062	1.467 ± 0.055	3.942 ± 0.027		5.617 ± 0.026
Water in sunlight:					
Blades 1 & 2.....	69.48 ± 0.062	2.229 ± 0.012	6.545 ± 0.003	2.632	9.118 ± 0.016
Blades 7 & 8.....	69.58 ± 0.281	2.233 ± 0.028	7.067 ± 0.013	3.125	9.673 ± 0.014
Glucose in darkness:					
Blades 1 & 2.....	70.89 ± 0.033	2.853 ± 0.001	6.154 ± 0.012	2.241	9.331 ± 0.014
Blades 7 & 8.....	70.01 ± 0.024	3.829 ± 0.039	6.827 ± 0.024	2.885	11.016 ± 0.064

2. *Time and the formation of sucrose:*

Detailed studies of time and the formation of sucrose from glucose or fructose have already been published (35). The hourly formation of sucrose in blades supplied with glucose is depicted in Fig. 1, and in blades supplied with fructose in Fig. 2. The percentage of cane sugar increased considerably more than the percentage of simple sugars, although the blades were supplied with simple sugars. These

EFFECT OF TIME UPON THE FORMATION OF CANE SUGAR BY SUGAR CANE SUPPLIED WITH GLUCOSE (5%)

Total Sugars

Cane Sugar

Simple Sugars

% SUGAR IN BLADES

TIME IN HOURS

Fig. 1

EFFECT OF TIME UPON THE FORMATION OF CANE SUGAR BY SUGAR CANE SUPPLIED WITH FRUCTOSE (5%)

Total Sugars

Cane Sugar

Simple Sugars

% SUGAR IN BLADES

TIME IN HOURS
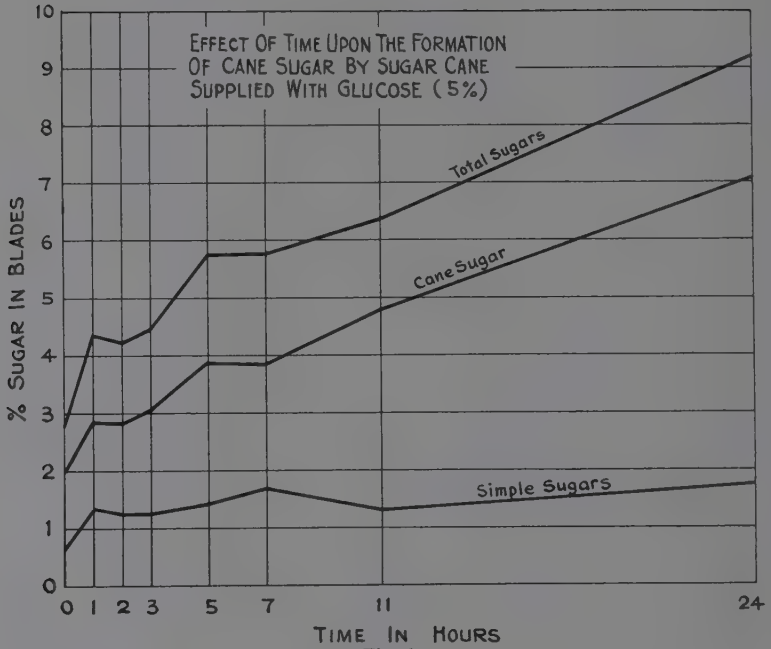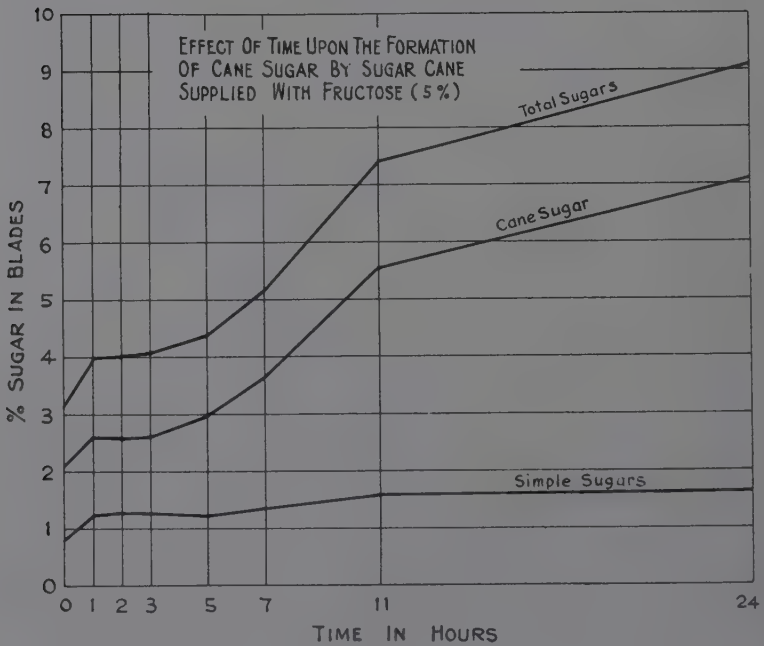
Fig. 2

curves are very similar to curves obtained in studies of fluctuations of sugars in attached blades in the open during the day and the night (32).

The daily formation of sucrose in sugar cane blades supplied with glucose is shown graphically in Fig. 3. The percentage of sucrose increased for nearly two weeks, yet the percentages of glucose and fructose showed only minor fluctuations.

THE DAILY FORMATION OF CANE SUGAR BY SUGAR CANE BLADES SUPPLIED WITH GLUCOSE

Fig. 3

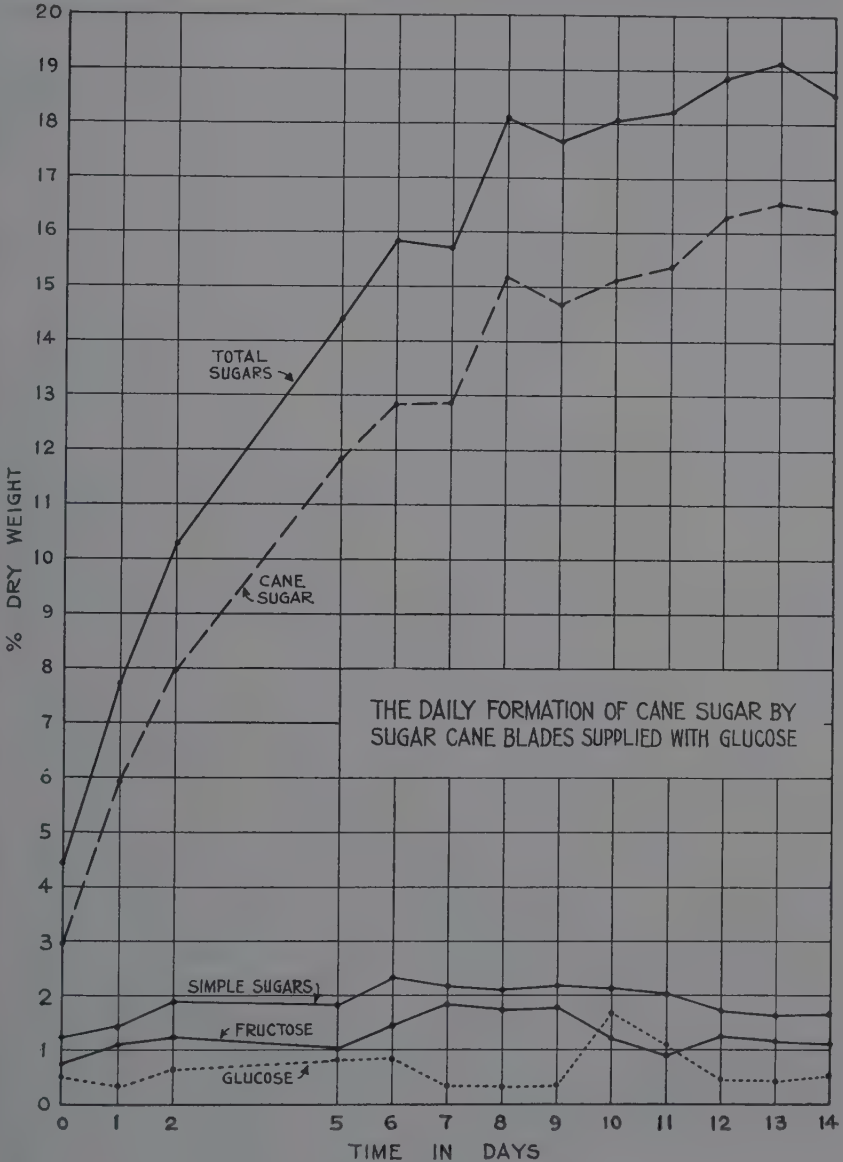The time of day when the leaves are taken from the plant is another factor affecting synthesis. In all routine tests the leaves are taken between eight and nine o'clock in the morning. To determine whether there is a diurnal fluctuation in the mechanism of synthesis, blades were taken one day at 9 a.m. and 9 p.m., and another day at 4:30 a.m. and 4:30 p.m. All the blades remained in 5 per cent glucose for exactly 24 hours. The results are presented in Table IX. The gain in total sugar and sucrose and the synthetic efficiency, calculated from Table IX, are shown in Table X. The formation of sucrose from glucose, fructose, or both glucose and fructose was least efficient in blades taken at 9 p.m. This was not due to lack of light, because blades taken at 4:30 a.m. were as efficient as blades taken in the daylight hours.

TABLE IX

MOISTURE AND SUGAR PERCENTAGES IN BLADES TAKEN AT DIFFERENT TIMES OF THE DAY AND SUPPLIED WITH 5% GLUCOSE OR FRUCTOSE FOR 24 HOURS

Series	Moisture	Reducing sugars	Sucrose	Total sugars
Initial control:				
9:00 a.m.	72.38 ± 0.143	0.929 ± 0.010	2.902 ± 0.016	3.983 ± 0.006
4:30 p.m.	70.58 ± 0.110	1.098 ± 0.017	5.145 ± 0.027	6.514 ± 0.045
9:00 p.m.	72.05 ± 0.038	1.017 ± 0.010	4.034 ± 0.027	5.263 ± 0.018
4:30 a.m.	72.09 ± 0.057	1.041 ± 0.012	2.704 ± 0.013	3.888 ± 0.027
Supplied with glucose:				
9:00 a.m.	70.85 ± 0.038	1.525 ± 0.001	6.091 ± 0.001	7.937 ± 0.003
4:30 p.m.	69.69 ± 0.019	1.395 ± 0.013	7.258 ± 0.008	9.035 ± 0.005
9:00 p.m.	72.13 ± 0.119	1.650 ± 0.003	5.777 ± 0.001	7.731 ± 0.005
4:30 a.m.	69.92 ± 0.086	1.359 ± 0.001	5.000 ± 0.002	6.622 ± 0.003
Supplied with fructose:				
9:00 a.m.	71.08 ± 0.110	1.296 ± 0.008	4.942 ± 0.024	6.499 ± 0.017
4:30 p.m.	69.12 ± 0.024	1.500 ± 0.000	7.410 ± 0.008	9.300 ± 0.008
9:00 p.m.	71.58 ± 0.086	1.473 ± 0.006	6.021 ± 0.011	7.811 ± 0.006
4:30 a.m.	70.39 ± 0.043	1.283 ± 0.026	5.238 ± 0.018	6.798 ± 0.045
Supplied with glucose and fructose:				
9:00 a.m.	69.84 ± 0.091	1.138 ± 0.002	5.677 ± 0.011	7.114 ± 0.014
4:30 p.m.	68.19 ± 0.024	1.386 ± 0.010	7.598 ± 0.021	9.384 ± 0.033
9:00 p.m.	71.33 ± 0.000	1.320 ± 0.006	6.025 ± 0.004	7.663 ± 0.010
4:30 a.m.	70.51 ± 0.000	1.255 ± 0.003	4.799 ± 0.012	6.307 ± 0.010

TABLE X

GAINS IN SUGARS AND SYNTHETIC EFFICIENCY IN BLADES TAKEN AT DIFFERENT TIMES OF THE DAY AND SUPPLIED WITH 5% GLUCOSE OR FRUCTOSE FOR 24 HOURS, CALCULATED FROM TABLE IX

Series	Gain in total sugar %	Gain in sucrose %	Synthetic efficiency
Supplied with glucose:			
9:00 a.m.	3.954	3.189	80.65
4:30 p.m.	2.521	2.113	83.81
9:00 p.m.	2.468	1.743	70.62
4:30 a.m.	2.734	2.296	83.97
Supplied with fructose:			
9:00 a.m.	2.516	2.040	81.08
4:30 p.m.	2.786	2.265	81.29
9:00 p.m.	2.548	1.987	77.98
4:30 a.m.	2.910	2.534	87.07

Supplied with glucose + fructose:

9:00 a.m.	3.131	2.775	88.62
4:30 p.m.	2.870	2.453	85.47
9:00 p.m.	2.400	1.991	82.95
4:30 a.m.	2.419	2.095	86.60

The percentages of glucose and fructose are recorded in Table XI which shows that the series given glucose at 9 a.m. made fructose, but the series given glucose at 9 p.m. accumulated glucose and lost fructose. It would seem that the blades taken

TABLE XI

FRUCTOSE AND GLUCOSE PERCENTAGES IN BLADES TAKEN AT DIFFERENT TIMES OF THE DAY AND SUPPLIED WITH 5% GLUCOSE OR FRUCTOSE FOR 24 HOURS

Series	Fructose	Gain in fructose	Glucose	Gain in glucose
Initial control:				
9 a.m.	0.764 ± 0.005		0.165 ± 0.005	
9 p.m.	0.652 ± 0.022		0.365 ± 0.012	
Supplied with glucose:				
9 a.m.	1.004 ± 0.066	0.240	0.521 ± 0.065	0.356
9 p.m.	0.484 ± 0.025	—0.168	1.166 ± 0.021	0.801
Supplied with fructose:				
9 a.m.	0.653 ± 0.025	—0.111	0.643 ± 0.034	0.478
9 p.m.	0.649 ± 0.001	—0.003	0.823 ± 0.007	0.458
Supplied with glucose + fructose:				
9 a.m.	0.582 ± 0.048	—0.182	0.556 ± 0.051	0.391
9 p.m.	0.998 ± 0.004	0.346	0.325 ± 0.004	—0.040

at 9 p.m. had difficulty in converting glucose to fructose. When given fructose, however, they made glucose, which may explain why their synthetic efficiency when given fructose was higher than when given glucose at 9 p.m.

These results suggest that some component of the mechanism of conversion of glucose to fructose and formation of sucrose is less active in blades detached from the plant at night than in blades detached from the plant during the day, but that this component is active again in blades detached from the plant in the early morning before dawn. Darkness itself is not the controlling factor, because all these tests are conducted in total darkness, and the results in Fig. 3 show that blades can continue to make sucrose from glucose when kept in total darkness for two weeks. This component may be used up or bound during the day and resupplied or released during the night, or there may be a "circulation" of the component into and out of the blade. In this connection the diurnal migration of phosphorus from bean leaves studied by Biddulph (4) is highly suggestive. Biddulph found no migration of phosphorus from 7 p.m. to midnight, and that the migration of phosphorus both into and out of the leaf was accelerated in the early morning before dawn.

3. *Temperature and the formation of sucrose:*

Detailed studies of the effect of temperature upon the interconversion of glucose and fructose and the formation of sucrose have already been published in full (35). The effect of temperature upon the daily percentages of sugars in sugar cane blades supplied with glucose is graphed in Fig. 4, and in blades supplied with fructose in Fig. 5. At 6°C. the blades absorbed the sugar supplied but there was little conver-

sion to the other reducing sugar and little formation of sucrose. But at 20°C. most of the sugar absorbed was converted into sucrose. At 30°C. and 40°C. there was a greater accumulation of sucrose than at the lower temperatures, and also both glucose and fructose accumulated. The higher temperatures increased the absorption of sugar, as shown by the percentages of total sugars. The synthetic efficiency was
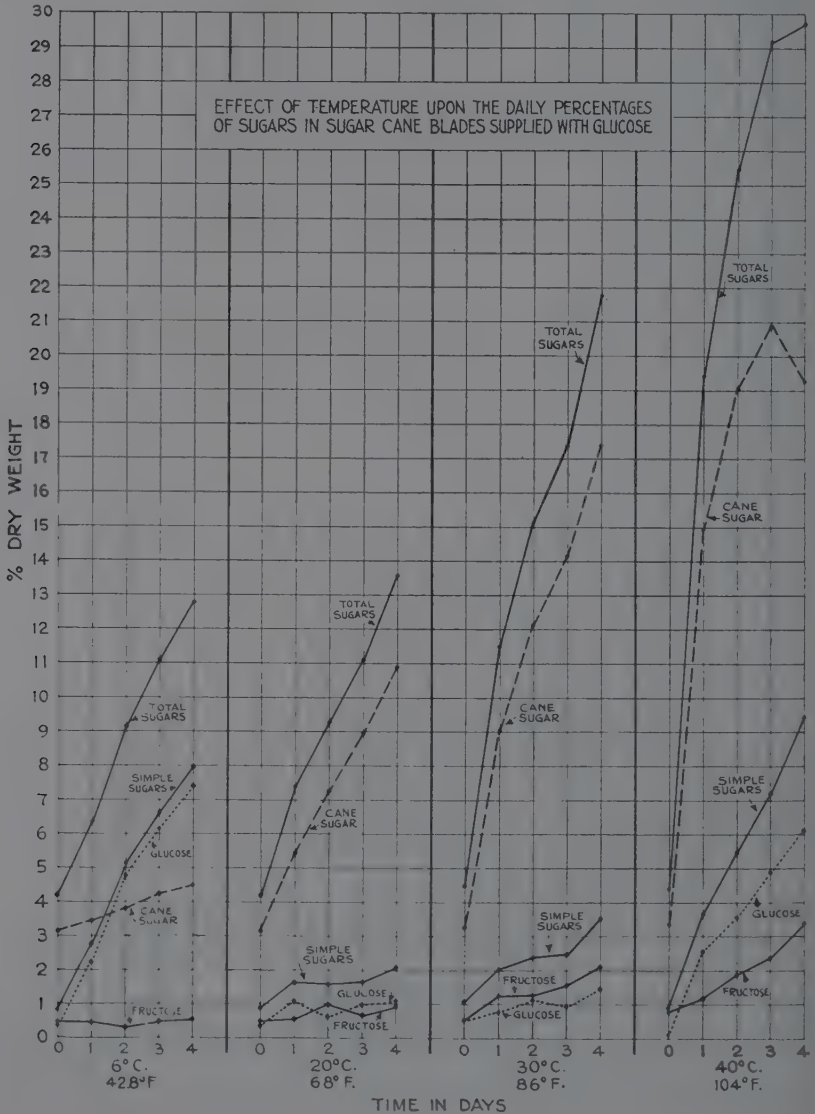

Fig. 4

best at 30°C. These results show that temperature affects the absorption of sugar, the interconversion of glucose and fructose, and the formation of sucrose.

4. *Chlorophyll and the formation of sucrose:*

Partially and completely albino stalks occasionally develop in stools of cane in the field. One completely albino stalk and one green stalk from the same stool of cane, variety POJ 2878, were used in a test reported in 1937 (33), which showed that albino blades can make sucrose from glucose exactly as well as green blades.
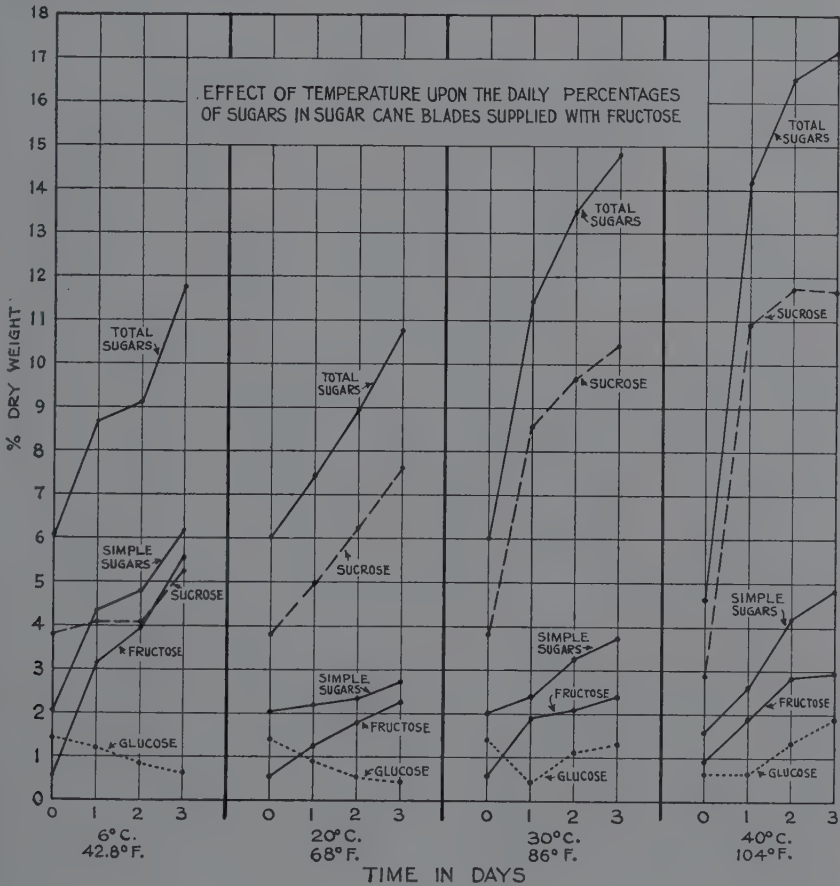

Fig. 5

This experiment was repeated using blades from six albino stalks and six green stalks, using cross No. 5128. In one test the blades were supplied with glucose and in another test, using ratoons, they were supplied with fructose. The results are presented in Table XII. The synthetic efficiencies of the blades supplied with glucose were as follows: albino, 70.74; green, 55.61. The synthetic efficiencies of the blades

TABLE XII

MOISTURE AND SUGAR PERCENTAGES IN ALBINO AND GREEN BLADES
OF CROSS NO. 5128, SUPPLIED WITH GLUCOSE OR FRUCTOSE FOR 24 HOURS

Series	Moisture	Reducing sugars	Sucrose	Total sugars
Initial control:				
Albino	79.98 ± 0.224	0.975 ± 0.040	1.092 ± 0.030	2.124 ± 0.008
Green	77.06 ± 0.200	1.029 ± 0.005	2.027 ± 0.022	3.163 ± 0.028
Supplied with glucose:				
Albino	78.25 ± 0.219	3.537 ± 0.011	7.150 ± 0.065	10.687 ± 0.053
Green	76.14 ± 0.358	6.020 ± 0.084	8.723 ± 0.035	15.202 ± 0.121
Initial control:				
Albino	71.20 ± 0.029	0.952 ± 0.003	1.311 ± 0.001	2.332 ± 0.003
Green	68.58 ± 0.057	0.557 ± 0.033	4.630 ± 0.035	5.431 ± 0.004
Supplied with fructose:				
Albino	68.65 ± 0.296	2.005 ± 0.031	2.772 ± 0.076	4.923 ± 0.049
Green	63.91 ± 0.224	2.966 ± 0.014	5.012 ± 0.033	8.242 ± 0.049

supplied with fructose were as follows: albino, 56.39; green, 13.59. The albino blades made sucrose even better than the green blades.

The synthetic efficiencies of the green blades are the lowest ever obtained with apparently normal green blades in the absence of inhibitory substances. No explanation can be offered except that a new cross was used.

Since blades totally lacking in chlorophyll can make sucrose from either glucose or fructose, it is obvious that chlorophyll plays no part in the synthesis of sucrose. Roots are also totally devoid of chlorophyll, but excised roots readily make sucrose when aerated, a point to be discussed in another section. Since neither chlorophyll nor light is required for the formation of sucrose, the process of the synthesis of sucrose must be distinct from photosynthesis, which, however, is essential for it supplies the raw materials for the formation of sucrose.

In another experiment, etiolated shoots of variety H 109 were used instead of albino blades. The percentages of moisture and sugars are presented in Table XIII, the gains in sugars and the synthetic efficiencies in Table XIV, and the percentages of fructose and glucose in Table XV. It is true that the etiolated shoots made

TABLE XIII

MOISTURE AND SUGAR PERCENTAGES IN ETIOLATED SHOOTS SUPPLIED
WITH GLUCOSE OR FRUCTOSE FOR 24 HOURS

Series	Moisture	Reducing sugars	Sucrose	Total sugars
Initial control	88.78 ± 0.009	10.142 ± 0.092	3.15 ± 0.052	13.46 ± 0.038
Glucose	86.70 ± 0.253	11.203 ± 0.077	4.45 ± 0.000	15.88 ± 0.076
Fructose	86.98 ± 0.081	11.799 ± 0.006	3.69 ± 0.009	15.69 ± 0.019

TABLE XIV

GAINS IN SUGARS AND SYNTHETIC EFFICIENCY IN ETIOLATED SHOOTS
SUPPLIED WITH GLUCOSE OR FRUCTOSE FOR 24 HOURS,
CALCULATED FROM TABLE XIII

Series	Gain in total sugars	Gain in sucrose	Synthetic efficiency
Glucose	2.42	1.30	53.71
Fructose	2.23	0.54	24.21

TABLE XV

FRUCTOSE AND GLUCOSE PERCENTAGES IN ETIOLATED SHOOTS SUPPLIED WITH GLUCOSE OR FRUCTOSE FOR 24 HOURS

Series	Fructose	Gain in fructose	Glucose	Gain in glucose
Initial control	4.592 ± 0.000		5.550 ± 0.092	
Glucose	5.497 ± 0.031	0.905	5.706 ± 0.046	0.156
Fructose	5.112 ± 0.029	0.520	6.686 ± 0.035	1.136

sucrose. However, the synthetic efficiencies with either glucose or fructose were lower than generally obtained with variety H–109 under normal conditions.

The results for fructose and glucose are of considerable interest, for in the shoots supplied with glucose there was an accumulation of fructose; and in the shoots supplied with fructose there was an accumulation of glucose. Evidently the interconversion of glucose and fructose could take place readily in these etiolated shoots.

Several attempts were made to repeat this experiment with etiolated shoots, but without success. The etiolated shoots were already so high in sugars (chiefly reducing sugars) that they seldom could be induced to take up more.

5. *Aeration and the formation of sucrose:*

The importance of aeration for interconversion and synthesis was studied in both blades and roots. The effect of aeration in blades was studied by submerging some of the blades in solutions of glucose or fructose. Air was supplied to the aerated series by being pumped through aerators. Several types of aerators were used, and the most satisfactory was found to be pressure tubing punctured uniformly 48 times with an ice pick. The amount of aeration was varied by using one or more aerators per series. The results of one of the experiments with blades are recorded in Table XVI. The gains in total sugars and sucrose and the synthetic efficiency are shown in Table XVII. The blades deprived of aeration absorbed some sugar but lost sucrose, while the blades which were aerated absorbed more sugar and made a little

TABLE XVI

MOISTURE AND SUGAR PERCENTAGES IN BLADES SUPPLIED WITH 5% GLUCOSE OR FRUCTOSE WITH AND WITHOUT AERATION

Series	Moisture	Reducing sugars	Sucrose	Total sugars
Initial control	68.10 ± 0.048	0.602 ± 0.025	2.205 ± 0.023	2.924 ± 0.001
Emergent blades:				
In glucose	68.42 ± 0.019	1.525 ± 0.023	6.503 ± 0.071	8.370 ± 0.052
In fructose	67.96 ± 0.052	1.701 ± 0.026	6.073 ± 0.027	8.094 ± 0.002
In both	68.08 ± 0.019	1.355 ± 0.016	6.125 ± 0.006	7.802 ± 0.022
Submerged—not aerated:				
In glucose	71.27 ± 0.019	2.108 ± 0.008	1.609 ± 0.035	3.802 ± 0.028
In fructose	70.51 ± 0.038	1.515 ± 0.016	1.792 ± 0.002	3.400 ± 0.013
In both	69.97 ± 0.048	1.279 ± 0.002	1.955 ± 0.012	3.338 ± 0.011
Submerged—aerated:				
In glucose	70.69 ± 0.043	1.273 ± 0.003	2.719 ± 0.025	4.135 ± 0.023
In fructose	71.59 ± 0.081	1.658 ± 0.000	3.157 ± 0.011	4.982 ± 0.011
In both	70.62 ± 0.048	1.367 ± 0.013	3.668 ± 0.005	5.223 ± 0.019

TABLE XVII

GAINS IN SUGARS AND SYNTHETIC EFFICIENCY OF BLADES SUPPLIED WITH
5% GLUCOSE OR FRUCTOSE WITH AND WITHOUT AERATION,
CALCULATED FROM TABLE XVI

Series	Gain in total sugars	Gain in sucrose	Synthetic efficiency
Emergent blades:			
In glucose	5.446	4.298	78.92
In fructose	5.170	3.868	74.81
In both	4.878	3.920	80.36
Submerged—not aerated:			
In glucose	0.878	—0.596	0
In fructose	0.476	—0.413	0
In both	0.414	—0.250	0
Submerged—aerated:			
In glucose	1.211	0.514	42.44
In fructose	2.058	0.952	46.25
In both	2.305	1.463	63.47

sucrose. Synthesis was not as efficient in the submerged, aerated blades as in the
emergent blades, as shown by the synthetic efficiency.

The effect of aeration upon synthesis in roots is shown in Table XVIII. The
gains in total sugars and sucrose and the synthetic efficiency are shown in Table
XIX. The excised roots with no aeration absorbed glucose but made no sucrose.

TABLE XVIII

MOISTURE AND SUGAR PERCENTAGES IN EXCISED ROOTS SUPPLIED WITH
5% GLUCOSE, WITH DIFFERENT AMOUNTS OF AERATION

Series	Moisture	Reducing sugars	Sucrose	Total sugars
Initial control	87.24 ± 0.086	1.402 ± 0.060	2.662 ± 0.007	4.204 ± 0.052
Glucose:				
No aerators	89.07 ± 0.133	6.590 ± 0.024	1.676 ± 0.023	8.355 ± 0.000
1 aerator	89.08 ± 0.157	7.873 ± 0.002	10.443 ± 0.076	18.866 ± 0.078
2 aerators	86.75 ± 0.215	6.621 ± 0.040	11.726 ± 0.127	18.965 ± 0.093
3 aerators	86.80 ± 0.129	6.122 ± 0.026	13.218 ± 0.005	20.035 ± 0.030
6 aerators	86.13 ± 0.100	5.907 ± 0.016	13.064 ± 0.038	19.659 ± 0.056

TABLE XIX

GAINS IN SUGARS AND SYNTHETIC EFFICIENCY OF ROOTS SUPPLIED WITH
5% GLUCOSE, WITH DIFFERENT AMOUNTS OF AERATION,
CALCULATED FROM TABLE XVIII

Series	Gain in total sugars	Gain in sucrose	Synthetic efficiency
No aerators	4.151	—0.986	0
1 aerator	14.662	7.781	53.06
2 aerators	14.761	9.064	61.40
3 aerators	15.831	10.556	66.67
6 aerators	15.455	10.402	67.30

When aerated, they absorbed much more glucose and made considerable sucrose.
Increasing the number of aerators from one to three increased both absorption and
synthesis, but increasing the number of aerators from three to six had very little
effect.

The results for glucose and fructose are reported in Table XX. In the series

TABLE XX

FRUCTOSE AND GLUCOSE PERCENTAGES IN EXCISED ROOTS SUPPLIED WITH
5% GLUCOSE, WITH DIFFERENT AMOUNTS OF AERATION

Series	Fructose	Gain in fructose	Glucose	Gain in glucose
Initial control	1.160 ± 0.160		0.241 ± 0.101	
No aerators	1.709 ± 0.018	0.549	4.881 ± 0.006	4.640
1 aerator	1.865 ± 0.040	0.705	6.008 ± 0.039	5.767
2 aerators	2.421 ± 0.061	1.261	4.200 ± 0.101	3.959
3 aerators	2.255 ± 0.019	1.095	3.866 ± 0.045	3.625
6 aerators	2.698 ± 0.048	1.538	3.209 ± 0.031	2.968

with no aerators, all of the glucose absorbed remained as glucose in the roots, since the small gain in fructose could be accounted for by the loss in sucrose. With aeration there were greater gains in fructose as well as considerable gains in glucose. Evidently aeration is essential for the conversion of glucose to fructose. Aeration is required not only for the conversion of glucose to fructose but also for the synthesis of sucrose, since blades supplied with both glucose and fructose made no sucrose unless aerated. The exact rôle of aeration is not apparent from this test. Aeration may be needed for the formation of an intermediate compound, or for the release of energy required for synthesis, or both. This question will be discussed later.

Oxygen may be supplied to roots by the use of hydrogen peroxide, without forced aeration. Zimmerman (85) found that hydrogen peroxide is good for rooting cuttings. In the following experiment, 60 cc. of 3 per cent hydrogen peroxide were used per liter of 5 per cent glucose. The results are recorded in Table XXI. The gains in sugars and the synthetic efficiency are shown in Table XXII. Some

TABLE XXI

5% GLUCOSE AND OXYGEN FROM H_2O_2

MOISTURE AND SUGAR PERCENTAGES IN EXCISED ROOTS SUPPLIED WITH

Series	Moisture	Reducing sugars	Sucrose	Total sugars
Initial control	89.05 ± 0.091	2.707 ± 0.009	3.034 ± 0.030	5.900 ± 0.040
Glucose—3 aerators	86.88 ± 0.105	5.859 ± 0.019	14.845 ± 0.022	21.486 ± 0.042
Glucose—H_2O_2	89.53 ± 0.143	9.939 ± 0.022	4.151 ± 0.000	14.309 ± 0.022
Glucose—H_2O_2	89.58 ± 0.243	9.851 ± 0.040	4.214 ± 0.019	14.287 ± 0.019

TABLE XXII

GAINS IN SUGARS AND THE SYNTHETIC EFFICIENCY IN EXCISED ROOTS
SUPPLIED WITH 5% GLUCOSE AND OXYGEN FROM H_2O_2,
CALCULATED FROM TABLE XXI

Series	Gain in total sugars	Gain in sucrose	Synthetic efficiency
Glucose—3 aerators	15.586	11.811	75.77
Glucose—H_2O_2	8.409	1.117	13.28
Glucose—H_2O_2	8.387	1.180	14.06

sugar was absorbed and some sucrose made by the roots supplied with oxygen from hydrogen peroxide, but neither absorption nor synthesis was as good as when oxygen was supplied by forced aeration.

To recapitulate, aeration increases the absorption of glucose and is absolutely essential for the conversion of glucose into fructose and for the synthesis of sucrose.

6. *Mutilation and the formation of sucrose:*

The object of these tests was to find the effects of cutting and grinding upon the formation of sucrose from glucose. In the first experiment the blades were divided into thirds—the lower, middle, and upper third—and were then placed with their lower ends in 5 per cent glucose for 24 hours. The results are presented in Table XXIII. The gains in sugars and the synthetic efficiencies are recorded in Table XXIV which shows that the lower third of the blade was the most efficient in synthesis and the upper third of the blade absorbed the most sugar. The middle third of the blade was the poorest in both absorption and synthesis.

TABLE XXIII

MOISTURE AND SUGAR PERCENTAGES IN LOWER, MIDDLE, AND UPPER THIRDS OF BLADES, SUPPLIED WITH 5% GLUCOSE FOR 24 HOURS

Series	Moisture	Reducing sugars	Sucrose	Total sugars
Initial control:				
Entire	74.15 ± 0.172	1.338 ± 0.005	2.037 ± 0.001	3.483 ± 0.003
Lower third	77.84 ± 0.062	2.008 ± 0.011	3.420 ± 0.001	5.608 ± 0.012
Middle third	73.45 ± 0.215	1.125 ± 0.027	1.626 ± 0.008	2.837 ± 0.017
Upper third	68.73 ± 0.038	0.802 ± 0.008	1.003 ± 0.002	1.859 ± 0.010
In 5% glucose:				
Entire	71.07 ± 0.091	2.259 ± 0.006	6.484 ± 0.022	9.085 ± 0.017
Lower third	77.72 ± 0.200	2.127 ± 0.029	5.385 ± 0.009	7.796 ± 0.019
Middle third	71.54 ± 0.057	$1.637 \quad ....$	$2.553 \quad ....$	$4.324 \quad ....$
Upper third	66.35 ± 0.091	2.683 ± 0.011	6.219 ± 0.003	9.230 ± 0.015

TABLE XXIV

GAINS IN SUGARS AND SYNTHETIC EFFICIENCIES OF LOWER, MIDDLE, AND UPPER THIRDS OF BLADES SUPPLIED WITH 5% GLUCOSE FOR 24 HOURS, CALCULATED FROM TABLE XXIII

Series	Gain in total sugars	Gain in sucrose	Synthetic efficiency
Entire	5.602	4.447	79.38
Lower third	2.188	1.965	89.81
Middle third	1.487	0.927	62.34
Upper third	7.371	5.216	70.76

The effect of dividing the blade into midribs and laminae was next studied, with the results recorded in Table XXV. The gains in sugars and the synthetic efficiencies are tabulated in Table XXVI which shows that the laminae are more efficient in both absorption and synthesis than the midribs. The percentages of fructose and glucose are reported in Table XXVII which shows a tendency toward the accumulation of glucose in the midribs but not in the laminae. This indicates that the conversion of glucose to fructose takes place better in the laminae than in the midribs.

TABLE XXV

MOISTURE AND SUGAR PERCENTAGES IN MIDRIBS AND LAMINAE SUPPLIED
WITH 5% GLUCOSE FOR 24 HOURS

Series	Moisture	Reducing sugars	Sucrose	Total sugars
Initial control:				
Entire	72.20 ± 0.000	0.970 ± 0.000	1.644 ± 0.003	2.701 ± 0.004
Midribs	74.71 ± 0.172	1.516 ± 0.005	2.115 ± 0.018	3.742 ± 0.013
Laminae	67.99 ± 0.005	0.490 ± 0.023	1.120 ± 0.026	1.670 ± 0.004
In 5% glucose:				
Entire	71.02 ± 0.024	1.298 ± 0.001	5.133 ± 0.025	6.702 ± 0.027
Midribs	73.95 ± 0.019	2.732 ± 0.007	4.845 ± 0.011	7.832 ± 0.004
Laminae	67.52 ± 0.129	1.220 ± 0.018	5.753 ± 0.020	7.277 ± 0.003

TABLE XXVI

GAINS IN SUGARS AND SYNTHETIC EFFICIENCIES OF MIDRIBS AND LAMINAE
SUPPLIED WITH 5% GLUCOSE FOR 24 HOURS,
CALCULATED FROM TABLE XXV

Series	Gain in total sugars	Gain in sucrose	Synthetic efficiency
Entire	4.001	3.489	87.20
Midribs	4.090	2.730	66.74
Laminae	5.607	4.633	82.62

TABLE XXVII

FRUCTOSE AND GLUCOSE PERCENTAGES IN MIDRIBS AND LAMINAE SUPPLIED
WITH 5% GLUCOSE FOR 24 HOURS

Series	Fructose	Gain in fructose	Glucose	Gain in glucose
Initial control:				
Entire	0.344 ± 0.011		0.626 ± 0.010	
Midribs	0.862 ± 0.023		0.654 ± 0.017	
Laminae	0.463 ± 0.004		0.042 ± 0.020	
In 5% glucose:				
Entire	1.202 ± 0.020	0.858	0.096 ± 0.019	—0.530
Midribs	1.076 ± 0.051	0.214	1.656 ± 0.058	1.002
Laminae	0.674 ± 0.058	0.211	0.546 ± 0.040	0.504

The effect of cutting the blades into pieces approximately one inch in length was next studied. The blades were cut with scissors. Preliminary tests showed that blades cut in this manner and submerged in 5 per cent glucose with no aeration made little or no sucrose. Aeration enabled the cut blades to make sucrose, but the synthetic efficiency of the cut blades was not as great as that of the entire emergent blades. To find out whether it was cutting or submerging the blades that decreased synthesis, intact and cut blades, submerged, with and without aeration were then compared. The results are recorded in Table XXVIII. The gains in sugars and the synthetic efficiencies are reported in Table XXIX. Since the synthetic efficiency of the cut blades, aerated, was as high as that of the intact blades, aerated, it is evident that cutting the blades into pieces approximately the size of one inch does not decrease their ability to make sucrose from glucose.

Since cutting the blades did not decrease synthesis, the effect of grinding with the Buffalo Cutter was then studied. After 24 hours in 5 per cent glucose, aerated, the cut blades were washed in a wire basket, dried superficially by centrifuging, ground, and sampled. The ground blades were placed in cheesecloth in a wire

TABLE XXVIII

MOISTURE AND SUGAR PERCENTAGES IN INTACT AND CUT BLADES SUPPLIED WITH 5% GLUCOSE FOR 24 HOURS, WITH AND WITHOUT AERATION

Series	Moisture	Reducing sugars	Sucrose	Total sugars
Initial control	72.85 ± 0.071	1.586 ± 0.001	2.516 ± 0.011	4.234 ± 0.013
In 5% glucose:				
Intact emergent	68.53 ± 0.057	2.677 ± 0.011	10.795 ± 0.013	14.041 ± 0.025
Intact submerged,				
not aerated	73.66 ± 0.081	2.631 ± 0.005	1.830 ± 0.001	4.557 ± 0.006
Intact submerged,				
aerated	72.96 ± 0.052	2.496 ± 0.012	4.745 ± 0.001	7.491 ± 0.013
Cut, not aerated	73.70 ± 0.071	3.077 ± 0.011	1.248 ± 0.092	4.391 ± 0.086
Cut, aerated	73.72 ± 0.009	2.246 ± 0.001	4.330 ± 0.011	6.804 ± 0.010

TABLE XXIX

GAINS IN SUGARS AND SYNTHETIC EFFICIENCIES IN INTACT AND CUT BLADES SUPPLIED WITH 5% GLUCOSE FOR 24 HOURS, WITH AND WITHOUT AERATION, CALCULATED FROM TABLE XXVIII

Series	Gain in total sugars	Gain in sucrose	Synthetic efficiency
Intact emergent	9.807	8.279	84.41
Intact, submerged, not aerated	0.323	—0.686	0
Intact, submerged, aerated ...	3.257	2.229	68.43
Cut, not aerated	0.157	—1.268	0
Cut, aerated	2.570	1.814	70.58

basket, washed, centrifuged in cheesecloth, ground further, and sampled. The results are presented in Table XXX. The gains in sugars and the synthetic efficiencies are shown in Table XXXI which shows that although the ground blades gained in total sugar they made absolutely no sucrose.

The suggestion arose that the ground blades may have made sucrose, but that the sucrose was removed by washing. To determine this point, a test was conducted in which the ground blades were not washed, but were analyzed along with all the glucose supplied. The results are presented in Table XXXII which shows that the ground blades made no sucrose.

TABLE XXX

MOISTURE AND SUGAR PERCENTAGES IN CUT AND GROUND BLADES SUPPLIED WITH 5% GLUCOSE FOR 24 HOURS WITH AERATION

Series	Moisture	Reducing sugars	Sucrose	Total sugars
Initial control	74.48 ± 0.153	1.409 ± 0.005	2.354 ± 0.005	3.888 ± 0.011
Cut, aerated	76.60 ± 0.119	2.871 ± 0.007	4.335 ± 0.007	7.435 ± 0.000
Ground, aerated	77.41 ± 0.029	4.135 ± 0.008	2.152 ± 0.018	6.400 ± 0.011

TABLE XXXI

GAINS IN SUGARS AND SYNTHETIC EFFICIENCIES OF CUT AND GROUND BLADES SUPPLIED WITH 5% GLUCOSE FOR 24 HOURS WITH AERATION, CALCULATED FROM TABLE XXX

Series	Gain in total sugars	Gain in sucrose	Synthetic efficiency
Cut	3.547	1.981	55.85
Ground	2.512	—0.202	0

TABLE XXXII

MOISTURE AND SUGAR PERCENTAGES IN ENTIRE AND GROUND BLADES,
AERATED, SUPPLIED WITH 5% GLUCOSE FOR 24 HOURS; THE GROUND
BLADES ANALYZED WITH THE GLUCOSE WITHOUT WASHING

Series	Moisture	Reducing sugars	Sucrose	Total sugars
Initial control	70.83 ± 0.029	0.898 ± 0.008	2.020 ± 0.007	3.024 ± 0.000
Entire	69.80 ± 0.014	1.662 ± 0.005	6.884 ± 0.005	8.909 ± 0.000
Ground	71.74 ± 0.181	8.711 ± 0.046	0.531 ± 0.025	9.271 ± 0.019

TABLE XXXIII

FRUCTOSE AND GLUCOSE PERCENTAGES IN CUT AND GROUND BLADES
SUPPLIED WITH 5% GLUCOSE FOR 24 HOURS, WITH AERATION

Series	Fructose	Gain in fructose	Glucose	Gain in glucose
Initial control	1.003 ± 0.006		0.406 ± 0.012	
Cut	1.112 ± 0.071	0.109	1.759 ± 0.079	1.353
Ground	0.724 ± 0.025	—0.279	3.411 ± 0.033	3.005

Fructose and glucose percentages in the cut and ground blades are reported in
Table XXXIII. The ground blades lost fructose and accumulated glucose, indi-
cating that grinding the blades interfered with the conversion of glucose to fructose.

Summary:

The formation of sucrose from glucose can take place in detached blades,
sheaths, stems, and roots of the sugar cane plant. Sucrose synthesis can also take
place in entire stalks.

More sucrose accumulated in the stems of entire stalks of cane with leaves
attached than in excised cane with leaves removed, indicating that both synthesis
in situ and translocation from the leaf may be involved in the storage of sucrose
in the stem.

When entire plants with roots attached were placed in solutions of glucose, the
roots absorbed glucose and accumulated sucrose, but in the tops, only the stems
gained a little sucrose.

Because the formation of sucrose from glucose or fructose takes place in abso-
lute darkness and does not require chlorophyll, the synthesis of sucrose is a process
distinct from photosynthesis.

Blades taken from different levels on the stem differ in their ability to make
sucrose from glucose. The same difference is shown when blades store sucrose as
a result of photosynthesis. This suggests that the synthesis of sucrose takes place
by the same mechanism whether the glucose is supplied artificially or naturally by
the process of photosynthesis.

In hourly studies of blades supplied with glucose or fructose, the percentages of
sucrose increased much more than the percentages of reducing sugars. In blades
supplied with glucose, the percentages of sucrose increased for nearly two weeks,
yet the percentages of reducing sugars fluctuated very little.

Some component of the mechanism of conversion of glucose to fructose and
formation of sucrose appears to be less active in blades detached from plants at
night than in blades detached from plants during the day. This component is active
again in blades detached from plants in the early morning before dawn.

Temperature affects the absorption of sugar, the interconversion of glucose and fructose, and the formation of sucrose. Low temperature (6° C.) prevented interconversion and synthesis but did not prevent absorption. Synthesis was most efficient at 30° C.

The formation of sucrose from glucose or fructose took place as well or even better in completely albino blades as in green blades.

Etiolated shoots when supplied with glucose accumulated fructose, and when supplied with fructose accumulated glucose, indicating that they could carry on the interconversion of glucose and fructose. The production of sucrose was less than usual, in etiolated shoots.

Aeration is essential for the interconversion of glucose and fructose and for the formation of sucrose. Aeration also increases the absorption of sugar.

The lower third of the blade was the most efficient in synthesis and the upper third absorbed the most sugar. The middle third of the blade was the poorest in both absorption and synthesis. The laminae were more efficient than the midribs in absorption of sugar, conversion of glucose to fructose, and synthesis of sucrose.

Cutting the blades into pieces approximately one inch in length did not decrease synthesis, but grinding the blades inhibited synthesis completely, and also inhibited the conversion of glucose to fructose.

Sugar Prices

96° CENTRIFUGALS FOR THE PERIOD
DECEMBER 16, 1942, TO MARCH 15, 1943

Date	Per pound	Per ton	Remarks
Dec. 16, 1942–Mar. 15, 1943........	3.74¢	$74.80	Philippines

THE HAWAIIAN PLANTERS' RECORD

H. L. LYON, *Editor*

EXPERIMENT STATION STAFF

H. L. LYON, Director

TABLE OF CONTENTS